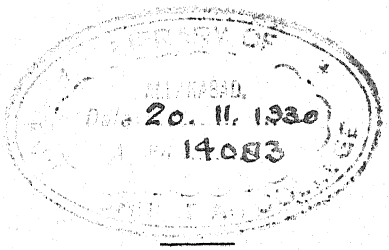
ALLAHABAD UNIVERSITY STUDIES

VOL. VI—PART II, (SCIENCE SECTION)

EDITED BY

The Vice-Chancellor and the Heads of Departments



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PREFACE

It has been found inconvenient to incorporate in the same volume matter dealing with the Science Section and the other sections. The Science Volume is therefore being sent out separately. It is a pity that we have not been able to secure any contributions from our Physics Department. It should not be understood however that the department is not carrying on research; because under the guidance of Professor Saha very good work is being done. Somehow no contributions could be sent in time to be included in the Volume.

We expect that this new arrangement will be found more convenient to our readers.

GANGANATHA JHA.

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THE CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF CERTAIN INDIAN TORTOISES

BY

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1. INTRODUCTION

The senior author has described in detail (1) the cytoplasmic inclusions in the oogenesis of the common European Tortoise, Testudo groeca. It was felt that a fairly exhaustive account of a more or less similar nature in the common North Indian Tortoise, Kachuga Smithii, would not be without interest. Among the Vertebrates the Tortoise appears to be the most suitable type for a beginner for the study of oogenesis. The follicular epithelium is single layered and the egg membranes when properly fixed appear as distinct structures. The formation of yolk is gradual and does not interfere with the study of the cell organs.

Among the previous workers on the subject only Bulliard (8) and A. Thing (21) need be mentioned.

Bulliard worked on the oogenesis of Emys lutaria. His work, however, loses its importance owing to the fact that he ignored the existence of Golgi bodies and thus lost sight of the importance which these cell organs play during oogenesis. Miss Thing confined her attention solely to the structure and function of the Zona pellucida in the ovarian eggs of Turtles. No other workers have so far tackled the Tortoises and Turtles for the study of cytoplasmic inclusions.

2. MATERIAL AND TECHNIQUE

Ovaries of fresh specimens of Kachuga Smithii from the local rivers and of specimens kept for months in our aquarium were examined for the sake of comparison. All possible precautions as recommended by Gatenby and Cowdry (12a), and Bowen (5) during the period of transference of the ovary from the body of the animal to the fixatives were taken. Among the fixatives which have given us satisfaction are the following :—Da Fano, Cajal, Ludford's latest Osmic method, Flemming without Acetic Acid, Champy, Flemming-Champy-Kolatchev modification, Regaud, Nassonov-Kolatchev, Ciaccio, Bouin, Dietrich aad Regaud-Tupa. As a rule, fixation in the cold at 0°C. gave us better results than at room temperature. Silver Nitrate preparations unless otherwise stated were treated in the ordinary way with Gold chloride and hypo. The best results were obtained by ·1% solution of Gold chloride, ·3% solution of Ammonium sulphocyanide, and 5% solution of hypo. Osmic preparations were bleached either by pure turpentine (impure turpentine like bad xylol washes out the Golgi bodies in no time) or by 1% solution of potassium permanganate and 4% solution of oxalic acid or by both.

3. THE GOLGI APPARATUS

The Golgi bodies in the early oocyte (Fig. 1) were found to mass together in a juxta-nuclear idiosome or yolk-nucleus

of Balbiani area. As has already been emphasised by so many workers before, the cytoplasm in this area is denser and takes in stain more readily, thus standing out in sharp contrast to the cytoplasm of the egg in general.

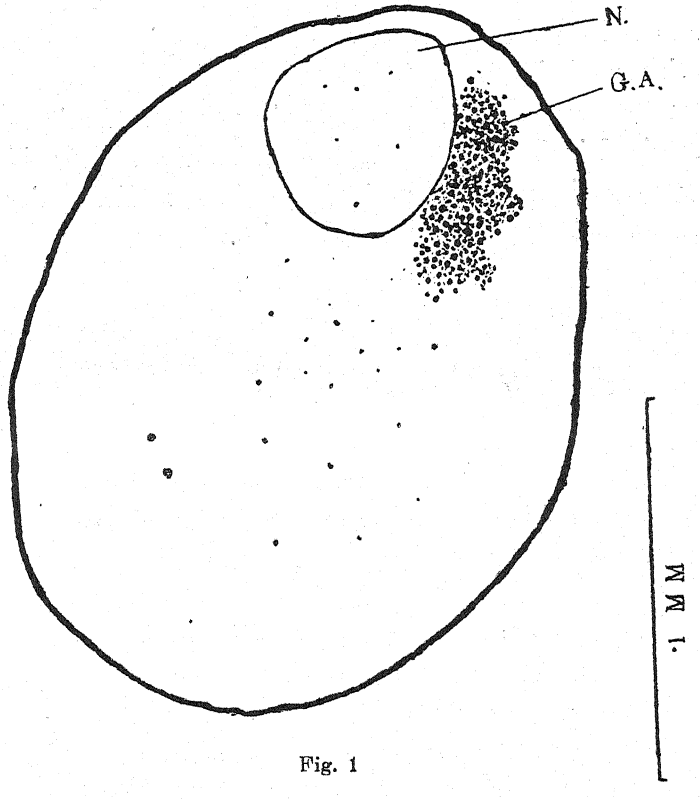

Fig. 1

Fig. 1. An early oocyte showing Golgi bodies massed together in a juxta-nuclear position. Osmic preparation by Ludford's method.

This stage is soon followed by one (Fig. 2), where the idiosome area increases in size and Golgi bodies become more distinct in structure and form a number of patches. By suitable methods of fixation and staining, it is possible to find in this area patches of Golgi bodies and mitochondria

side by side. For instance, in a Ludford preparation, properly bleached and stained by Kull's method, Golgi bodies appear as black spherical or granular bodies and Mitochondria as pinkish dust like particles in isolated patches and forming a semi-lunar cap around the nucleus.

Fig. 2 depicts a stage where the idiosome area has reached the maximum development and both the Golgi mass and the chondriome are breaking up into patches with a view to their final dispersal in the general cytoplasm. It may be mentioned here that fatty yolk formation (to be described in detail later) starts at this stage. By bleaching the slides carefully, it is possible to distinguish between the fatty yolk and Golgi bodies.

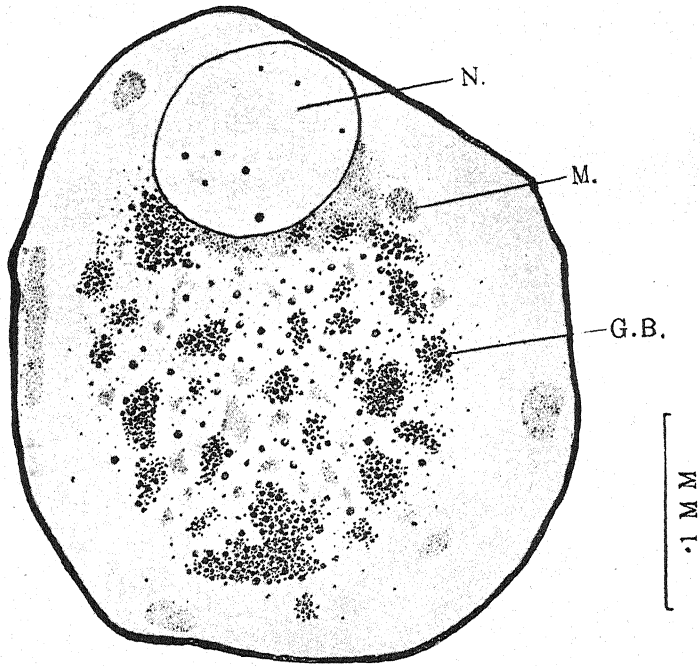

Fig. 2

Fig. 2. Golgi bodies and mitochondria in patches. Ludford method, bleached.

Fig. 3 depicts a later stage when the Golgi bodies after being distributed in the general cytoplasm in an haphazard

manner take up a definite position in the form of a ring-like peripheral zone. The process is not yet complete, as a portion of the original idiosome area still remains in tact. Yolk formation (F.Y.) begins in this area first and is taken up by the peripheral zone a little later.

It is noteworthy that at this stage the medullary region of the egg is practically free from Golgi bodies.

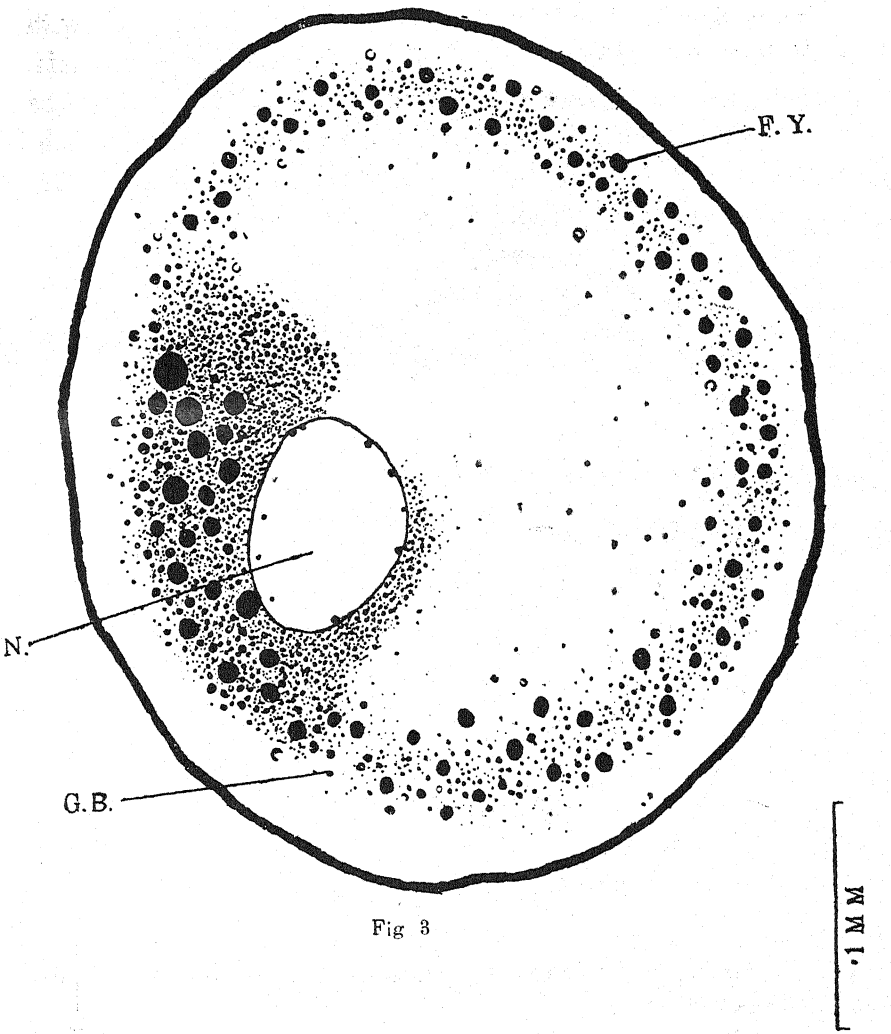

Fig 3

Fig. 3. Golgi bodies forming a peripheral ring-like zone. Yolk formation. Ludford, unbleached and unstained.

Fig. 4 depicts an advanced stage in the evolution of the Golgi bodies and the fatty yolk. The peripheral arrangement of Golgi bodies is lost and they are seen to be distributed all over the cytoplasm. Fatty yolk formation is now at its zenith and the egg is choked full of them. Golgi bodies as such are few and far between and have a tendency to become ultramicroscopic.

At about the stage when the peripheral ring of Golgi bodies is established, the egg membranes become sharply divided into the layers described by the senior author in

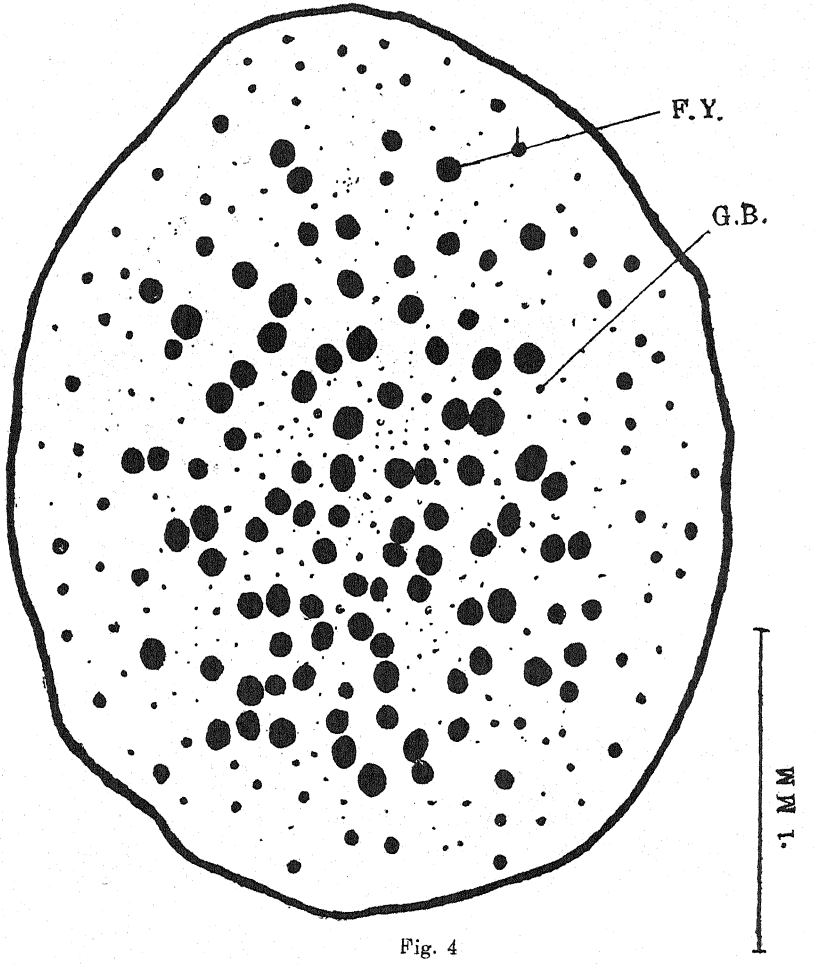

Fig. 4

Fig. 4. Golgi bodies and fatty yolk formation. Ludford, unbleached and unstained.

other animals (1, 3). A process of emigration of Golgi bodies from the follicular epithelium to the egg *via* the Zona radiata and the fibrillar layer can now easily be made out. These Golgi bodies settle down in the cortical region of the egg and are not distinguishable from the original Golgi elements which form the peripheral layer.

It appears as if the majority of the Golgi bodies has been used up in the formation of fatty yolk. The remaining ones probably break up and become ultramicroscopic. They appear to play no direct part in subsequent stages of development of the egg.

Fig. 5 represents a stained preparation as exactly seen

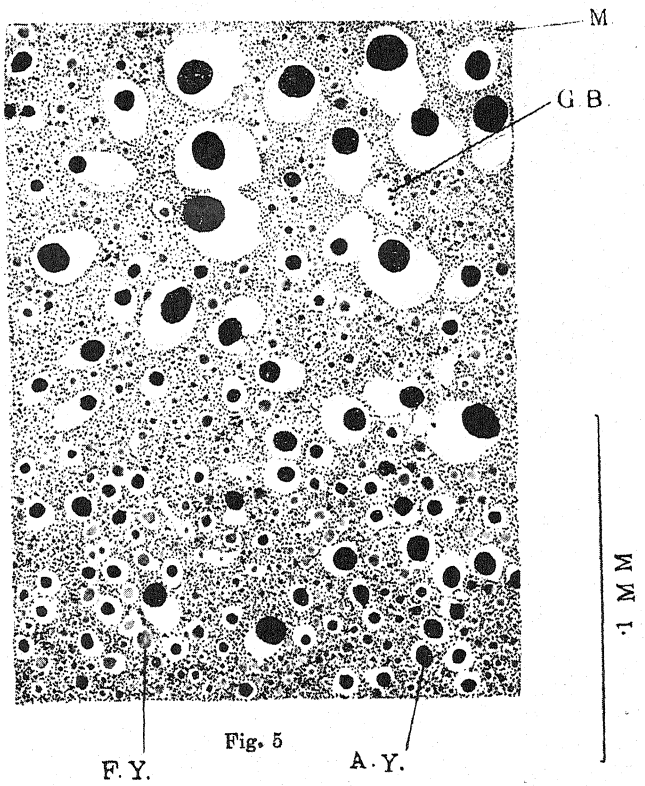

Fig. 5

Fig. 5. Formation of yolk and gradual disappearance of Golgi bodies. Flemming-Champy-Kolatchev modification Partially bleached and stained by Champy-Kull's method.

under the microscope. The greyish black Golgi bodies are difficult to make out, as most of them have by this time been utilised in the formation of fatty yolk (F.Y.).

4. THE MITOCHONDRIA

The mitochondria in the early stages form a juxta-nuclear mass in the same way as the Golgi bodies do. We are of opinion that both the Golgi bodies and mitochondria in the early stages of development of the oocyte occupy the area generally known as the idiosome or yolk-nucleus of Balbiani. This heart-shaped area functions as the focus of growth and dispersal both for the Golgi bodies and the mitochondria.

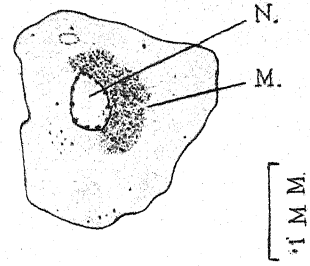

Fig. 6

Fig. 6. Mitochondria forming a juxta-nuclear mass in the idiosome area. Regaud-Tupa fixation. Champy-Kull stain.

Fig. 7 depicts a more advanced stage where the nucleus and the medullary region of the oocyte is surrounded by a very characteristic layer of dust-like mitochondria. The

central region or core as also the peripheral region of the egg are more or less free from mitochondria.

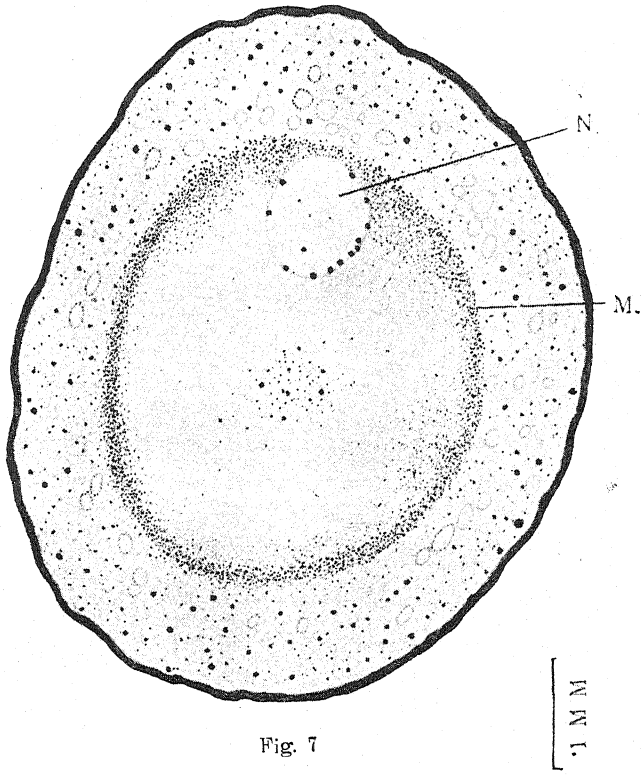

Fig. 7

Fig. 7. Mitochondria encircling the nucleus and medullary region of the egg. Regaud-Tupa fixation. Iron Alum and Hæmatoxylin stain.

This stage is transitory and is in a line with a similar distribution of Golgi bodies, which are however arranged in a more peripheral layer (Fig. 3).

The idiosome area has practically disappeared.

Fig. 8 depicts a stage where the mitochondria are distributed throughout the cytoplasm. Patches or plaquettes (M.P.) of mitochondria are distinctly visible.

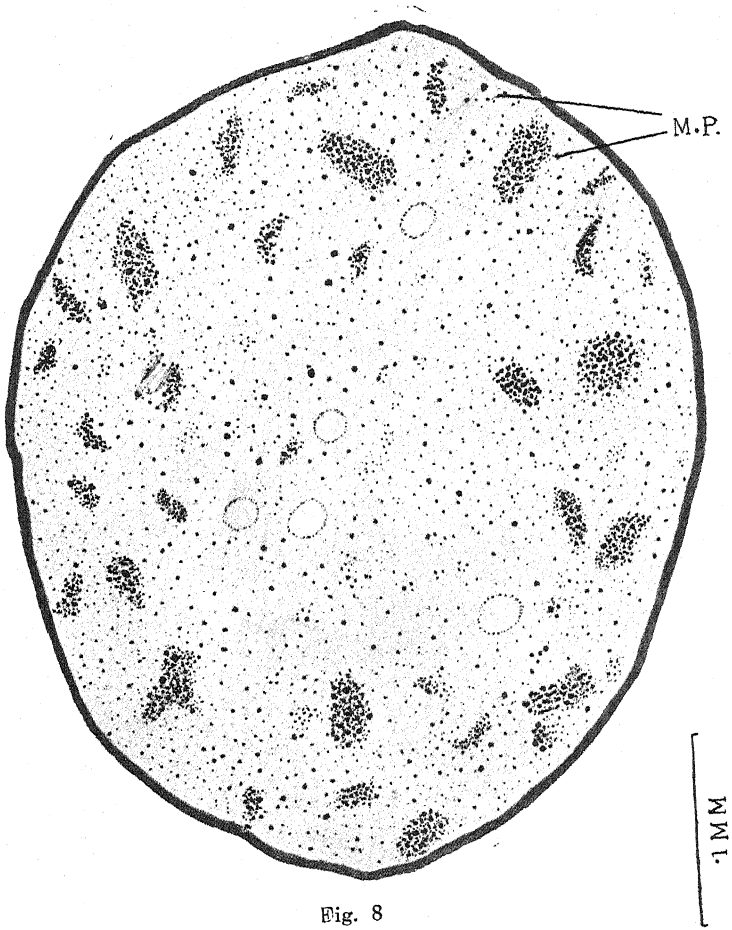

Fig. 8

Fig. 8. Mitochondria distributed throughout the cytoplasm. Patches or plaquettes (M.P.) of mitochondria still visible. Regaud-Tupa fixation. Champy-Kull stain.

This stage marks the beginning of the formation of albuminous yolk. The mitochondria seem to swell up and become transformed into yolk bodies.

In later stages (Fig. 9), the mitochondrial patches disappear and the mitochondria are evenly distributed throughout the cytoplasm. At this stage filamenter mito-

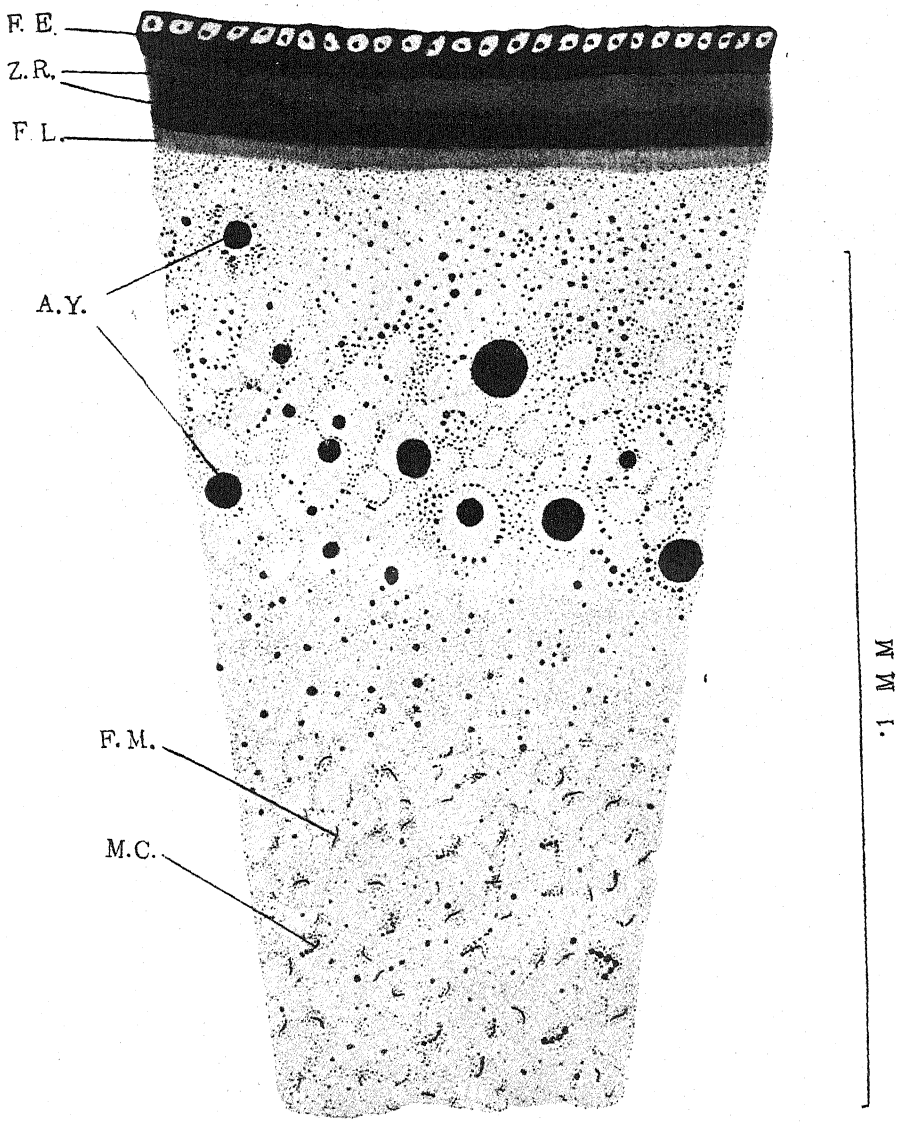

Fig. 9

Fig. 9 shows granular, beaded (M.C.) as well as filamenter (F.M.) mitochondria. Regaud fixation and post-chromisation for three months. Iron alum hæmatoxylin stain.

chondria (Fig. 9. F.M.) become visible in preparations made by methods advocated by Champy and Bulliard (9, 8). This consists in fixation by Regaud's method and then post-chromisation for three months and staining by Champy-Kull method or in iron alum hæmatoxylin.

The filaments take in a deep blue-black stain in hæmatoxylin. The spherical mitochondria in places appear to be arranged in beaded chains. The granular form of mitochondria is the commoner variety and most numerous. This stage is marked by great mitochondrial activity in the formation of yolk. A point of special interest which we wish to mention here casually is that as in the case of Golgi bodies (1, 3) mitochondria also appear to pass from the follicular epithelium to the egg *via* the Zona radiata. The senior author intends to describe this phenomenon in detail in a separate paper.

5. THE NUCLEOLAR EXTRUSIONS

The nucleolar extrusions in Kachuga are not such a marked feature as in Testudo groeca. The extrusions take no part in vitellogenesis and the process is apparently insignificant. The most effective way of demonstrating nucleolar extrusions is by Mann's methyl blue-eosine stain after Bouin fixation. The extrusions after emerging out of the nucleus lose their spherical form and power of colorability and are gradually absorbed in the general cytoplasm.

6. THE FORMATION OF YOLK BODIES

Two distinct kinds of yolk bodies are easily distinguishable—the fatty yolk and the albuminous yolk. The fatty yolk appears to arise through the intervention of Golgi bodies either directly or indirectly. The Golgi body swells up and comes to lie inside a vesicle. It draws the necessary requisites for its growth from the surrounding cytoplasm. In

rare cases a number of small Golgi bodies surround a vesicle and appear to play an accessory role in the formation of Golgi yolk. The Golgi bodies are thus solely responsible for the formation of fatty yolk. The fatty yolk is more easily blackened by osmic methods than the Golgi bodies. It is also more easily bleached than Golgi bodies. At the same time the fatty yolk is not to be confused with fat droplets which are easily extractable in turpentine and leave only a space behind. The fatty yolk is more resistible and even after it is bleached by turpentine and other methods, it leaves a dark rim behind, which indicates the remnant of the osmiophilic Golgi element. The Golgi yolk starts formation at a very early stage (Figs. 2 and 3). That this yolk is the outcome of the activities of Golgi bodies alone, is borne out by the facts that (1) it arises in the idiosome area inside Golgi patches, (2) the Golgi bodies disappear from view *pari passu* with the formation of fatty yolk, as growth proceeds.

The albuminous yolk in this animal is formed in two ways. Firstly, a mitochondrium swells up to more than double its normal size and is encircled by a vesicle. Growth proceeds inside the vesicle in the usual manner. According to the second method, the small granular mitochondria appear to surround a vesicle, in the middle of which yolk matter is deposited. Here the mitochondria take an indirect part in the formation of yolk. In the former case, the yolk is true mitochondrial yolk; in the latter, it is cytoplasmic yolk formed under the influence of mitochondria (Fig. 9). Figure 5 depicts a stage where both fatty yolk (F.Y.) and albuminous yolk (A. Y.) can be seen side by side. As may be seen, both kinds of yolk develop inside vesicles formed in the cytoplasm. When fully formed there is no way of distinguishing between mitochondrial yolk, and cytoplasmic yolk, as both of them follow the same fixing and staining reactions.

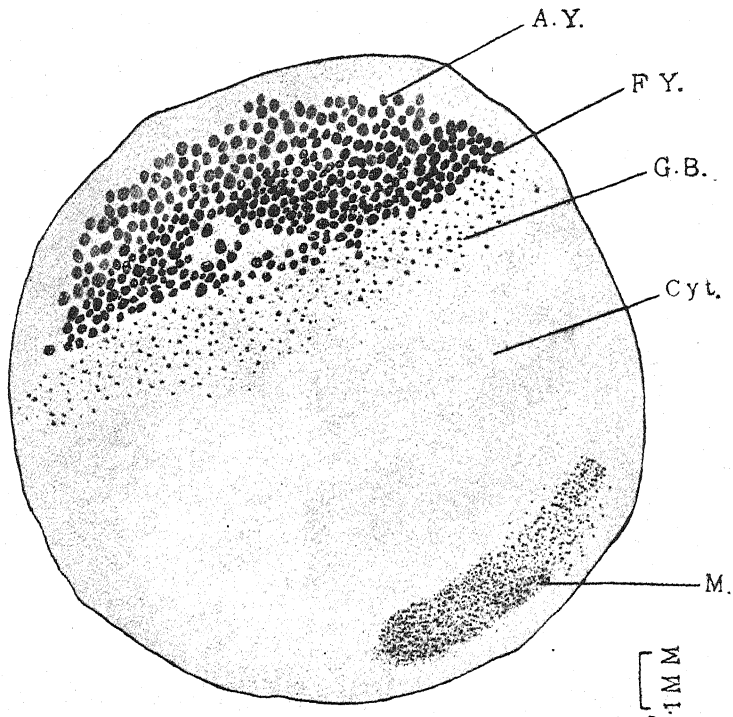

Fig. 10

Fig. 10 shows the result of centrifuging an egg at a velocity of 3,500 revolutions per minute. Champy-Kull.

7. THE CENTRIFUGE AND VITAL COLORATION EXPERIMENTS

Fig. 10 represents the result of centrifuging an egg at a velocity of 3,500 revolutions per minute for about a couple of hours. The material was then fixed in Champy's fluid. As might be expected, the yolk bodies lie at the top, the cytoplasm in the middle and the mitochondria at the bottom. In the close vicinity of fatty yolk lie some greyish black particles which we identify as Golgi bodies. The Champy-Kull stain gives the characteristic pink colour to the mass of mitochondria (M.) lying at the bottom.

Vital coloration experiments were performed both by the Neutral red (19, 2) and osmic acid (11) methods. The

latter was successful inasmuch as both the Golgi bodies and yolk got easily blackened. The former method did not succeed in spite of repeated attempts. Since the vital staining methods failed to give us satisfactory results we have not thought it advisable to discuss in this paper the supposed homology of the Golgi bodies and the vacuome.

8. DISCUSSION

Golgi bodies.--Stages of oocytes earlier than the one shown in Fig. 1 could not be found in spite of vigorous search. In Testudo groeca (1) earlier stages were found where Golgi bodies formed a sort of small Golgi complex in a juxta-nuclear position and were not discernible as separate entities till at a later stage. The Golgi bodies in Kachuga behave in the usual way, except in one important particular. Whereas in Testudo groeca the Golgi bodies take no part in the formation of yolk in Kachuga, the fatty yolk is formed solely by the Golgi bodies. Apart from the peripheral layer of comparatively larger Golgi bodies, a cortical layer of smaller Golgi granules is present close beneath the egg membrane as has been shown by the senior author previously (1, 3). The major portion of this layer is formed by Golgi bodies derived from the follicular epithelium by a process of infiltration through the Zona radiata.

Mitochondria.—As in the case of Golgi bodies, oocytes showing early mitochondrial stages could not be found. The mitochondria in this animal behave in much the same way as in Testudo groeca. The three zones of mitochondria —the perinuclear, the medullary and the cortical are present. Of these the medullary zone is very prominent and characteristic. As in Testudo groeca, the mitochondria metamorphose into mitochondrial yolk. Bulliard in Emys lutaria (Mars), describes the presence of filamenter mitochondria in all stages of development of the egg. We tried the methods advocated by him and succeeded in getting the

chondriocontes only at a particular stage in the development of the egg and that too only by Regaud's method, followed by post-chromisation for three months. The other mitochondrial and even the ordinary Regaud-Tupa methods failed to demonstrate filamenter mitochondria not only in this animal but also in all the vertebrates so far examined by the senior author. Naturally, therefore, we press the claim of the demonstration of filamenter mitochondria in this animal with some diffidence. Bulliard's claim needs confirmation by other workers, because if filamenter mitochondria could only be demonstrated in vertebrate oogenesis by prolonged post-chromisation, it must be admitted that an important cell organ is being overlooked by workers on this subject, for want of proper technique.

The yolk bodies.—Brambell (6) in 1924 described four types of yolk formation—the Golgi yolk, the mitochondrial yolk, the cytoplasmic yolk and the yolk formed by nucleolar extrusions. Gatenby and Nath (11) gave us the first hint as to the formation of two kinds of yolk essentially—the fatty yolk and the albuminous yolk. Since then Vishwa Nath in a series of papers has been emphasising the fact that the fatty yolk is derived directly or indirectly from Golgi bodies. The idea is not quite new for both Gatenby and Ludford had described previously the formation of yolk of a fatty nature from Golgi bodies. Recently, Hibbard (13) has described the formation of fatty yolk from Golgi bodies in the amphibian Discoglossus pictus, Otth. In Kachuga, the formation of fatty yolk from Golgi bodies leaves no doubt in our minds. The albuminous yolk in this animal is formed by (1) the direct metamorphosis of mitochondria into mitochondrial yolk, (2) the formation of a vesicle in the cytoplasm, which is surrounded by granular mitochondria and inside which yolk material is elaborated. The latter may be included under the category of cytoplasmic yolk formation.

9. SUMMARY

1. The Golgi bodies originate in the usual juxtanuclear position and form a peripheral layer in the later stages of development.

2. Fatty yolk is formed directly and indirectly by Golgi bodies and is abundant in the peripheral region of the egg. Fatty yolk formation begins much earlier than the albuminous yolk formation.

3. The cortical layer of Golgi bodies beneath the egg membrane is formed mostly of Golgi elements derived from the follicular epithelium by a process of infiltration through the Zona radiata.

4. The mitochondria also grow and multiply in the idiosome area, and later on become distributed in three zones in the egg—the perinuclear, the medullary and the cortical. The filamenter type of mitochondria has also been found after post-chromisation for three months.

5. The albuminous yolk is formed by (1) direct metamorphosis of mitochondria into a yolk body, (2) the formation of a vesicle which is encircled by small mitochondria and under the influence of which yolk matter is elaborated inside the vesicle.

6. Nucleolar extrusions take no part in the formation of yolk.

7. Centrifuge experiments separated the cell organs and yolk in the usual manner.

10. LETTERING

G. A. Golgi Apparatus in a mass, composed of small elements called Golgi bodies ;
N. Nucleus ;
G. B. Golgi bodies ;
M. Mitochondria ;
F. Y. Fatty yolk ;
A. Y. Albuminous yolk.
M. P. Mitochondrial patches or plaquettes of mitochondria

F. M. Filamenter mitochondria;
M. C. Beaded form of mitochondria;
F. L. Fibrillar layer;
Z. R. Zona radiata;
F. E. Follicular epithelium;
Cyt. Cytoplasm.

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NOTES ON CELL ORGANS IN THE OOGENESIS OF THE HOUSE GECKO

BY

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INTRODUCTION

The house gecko—Hemidactylus flaviviridis Rüppel—is the commonest type of wall lizard found in North India. The behaviour of its cell inclusions during oogenesis differs in several marked ways from those described in Calotes (6) and Uromastix (1). This note is a preliminary to a more detailed account which I intend to publish later. Unfortunately the ovary of this animal is comparatively very small and I have never succeeded in getting more than half a dozen oocytes in the ovarian tag, although I have examined over two dozen specimens. The procuring of successive stages has up to now been a matter of great difficulty. For this reason all stages could not be detailed in this paper.

The following fixatives were tried, but the best results were obtained by Da Fano, Ludford's latest osmic modification (10) and by Champy's fluid :—

1. Cajal's Uranium nitrate method.
2. Da Fano.
3. Kolachev-Nassonov.
4. Ludford.

5. Mann-Kopsch.
6. Champy.
7. Regaud-Tupa.
8. Bouin.

The usual methods of toning, bleaching and staining were adopted, as recommended in the *Vade Mecum*, edited by Gatenby and Cowdry.

I am deeply indebted to Dr. Bhattacharya for guidance and advice in the preparation of this paper. To Mr. S. K. Dutta, I offer my sincere thanks for procuring specimens for me and helping me in various other ways.

OBSERVATIONS

Golgi Bodies.—The Golgi bodies in the smallest oocytes available form as usual a complex mass in a juxta-nuclear position (Fig. 1). The extreme blackening that is caused at this stage both by silver nitrate and osmic methods does not allow of a close study of the component parts of the Golgi apparatus. It is therefore difficult to say whether the apparatus exists in the form of a reticulate mass or is formed by small spherical bodies run together into a mass under the influence of fixatives. In slightly older oocytes, the Golgi-tangle becomes more and more differentiated. At the stage when the yolk-nucleus of Balbiani or the idiosome area of Bowen is established, the Golgi bodies appear as discrete spherical, granular and crescent-shaped bodies (Figs. 1 and 2). This stage is followed by the dispersal stage, when the Golgi bodies become irregularly distributed in the cytoplasm either singly or in patches prior to their becoming arranged in a peripheral ring in advanced oocytes (Fig. 2). When this last stage is completed, the idiosome area disappears and very few Golgi bodies can be found outside the peripheral ring

(Fig. 3 P.R.). Ultimately, in still older oocytes, the peripheral area of Golgi bodies breaks up and gradually disappears from view as the vacuolar area (Fig. 3V) extends from the centre to the periphery. In more advanced stages, the Golgi bodies become ultramicroscopic and are lost in the general cytoplasm.

Prior to and even after the peripheral ring of Golgi bodies is established, it has been found that a regular process of infiltration of Golgi bodies from the follicular epithelium to the cortical region of the egg *via* the zona radiata, has been going on. Thus a fine but quite distinct layer of Golgi bodies is found in the extreme cortical region of the egg (Fig. 3 C.R.). Gecko, however, does not show the infiltration of Golgi bodies through channels in the zona radiata as described by Bhattacharya in the tortoise (1 and 3). The process is probably akin to that described by Brambell in the fowl (4).

Mitochondria.—In this animal the mitochondria are granular, spherical and rarely baton-shaped. In the early oocytes, the mitochondria form a cap-like investment at one pole of the nucleus. This cap develops into the yolk-nucleus of Balbiani area (Fig. 4 Y. N.). This stage is succeeded by the dispersal stage, where the mitochondria become distributed in the cytoplasm either singly or in patches (Fig. 5 M. P.). In a more advanced oocyte, the mitochondria for the greater part arrange themselves into a peripheral zone (Fig. 6 P. Z.) beneath the cortex of the egg. As all stages were not available, I am unable to say if a perinuclear and a medullary zone of mitochondria as are found in tortoises (1) and other animals (4) also exist in this animal. At about this stage vacuolar formations begin in the centre and medullary region of the egg.

Vacuoles.—The vacuoles described above are characteristic features of a developing oocyte and have also been described by Bhattacharya in uromastix (1). The lizards were

freshlycaught, which precluded the possibility of prolonged starvation. It appears that the ovary of this animal in the winter season, when this work was mostly done, goes through a resting stage. Even the largest oocytes show little sign of yolk formation. The medullary region becomes full of vacuolar growth and the cytoplasm and cell organs are pushed to the periphery of the egg. The nucleus shows no signs of nucleolar extrusions and it is difficult to say from the stages available, exactly how the yolk formation takes place. All that could be said is that in certain cases mitochondria are seen to swell up and form a vacuolar substances in their interior.

Post-Vital Experiments.—Ovaries were examined post-vitally in the neutral red solution as advised by Parat and Bhattacharya (12, 2) and also by the methods recommended by Gatenby and Nath (7). The vacuome was easily demonstrated by the former method and the Golgi bodies by the latter method. Whether both these structures are homologous, I am unable to say at the present moment. This controversial subject must be left open, till better technique and more convincing data are available.

Conclusion.—The Golgi bodies conform to the usual methods of origin, growth and distribution in the egg as have been described in several vertebrate forms by Gatenby, Hibbard (8), Brambell and Bhattacharya. In many invertebrate as well as vertebrate eggs, the Golgi bodies appear to give rise to fatty yolk (11). In this animal, however, they seem to play no part whatsoever in vitellogenesis. Neither Champy nor Ludford preparations show any signs of fat in the egg. In Calotes (6) Golgi bodies give rise to fatty yolk.

The infiltration of Golgi bodies from the follicular epithelium to the egg has been observed. Once these bodies settle down in the cortical region of the egg, they are difficult to distinguish from the original Golgi bodies of the egg.

The behaviour of mitochondria is practically the same as in other reptiles, except that in this case, there is only one zone. Only the peripheral zone of mitochondria has been found. In this zone, it has been observed that certain spherical mitochondria swell up to double the normal size and form a transparent core in their interior. Further growth gives them a vacuolar shape not very much unlike the vacuoles found in the egg.

In fairly well-grown oocytes, vacuoles are a marked feature of the medullary region of the egg. The vacuolar growth extends from the centre towards the periphery, and pushes out the cytoplasm and the cell organs towards the peripheral region of the egg. No signs of either fatty or albuminous yolk formation were visible.

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EXPLANATION OF PLATES

Fig. 1 shows an early oocyte where the Golgi apparatus is in the form of a close tangle in a juxta-nuclear position. Ludford preparation, bleached.

Fig. 2 shows the remains of yolk-nucleus of Balbiani. The Golgi bodies are becoming distributed in the Cytoplasm with a tendency to form a peripheral ring. Ludford preparation, bleached.

PLATE I

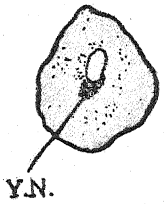

Fig. 1

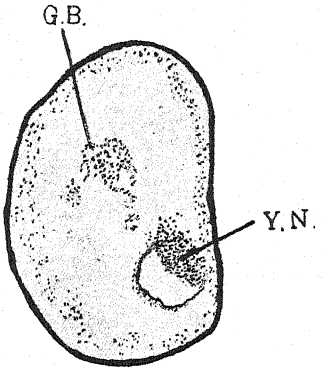

Fig. 2

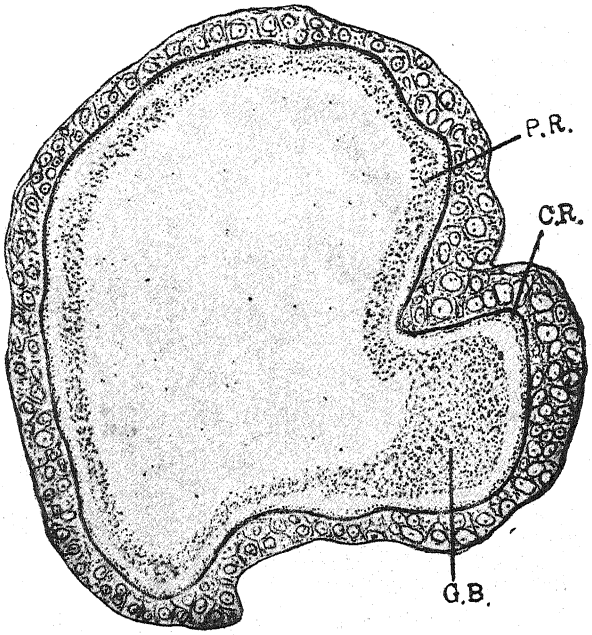

Fig. 3

PLATE II

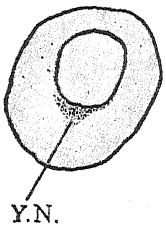

Fig. 4

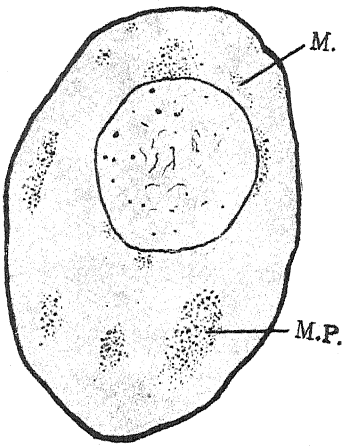

Fig. 5

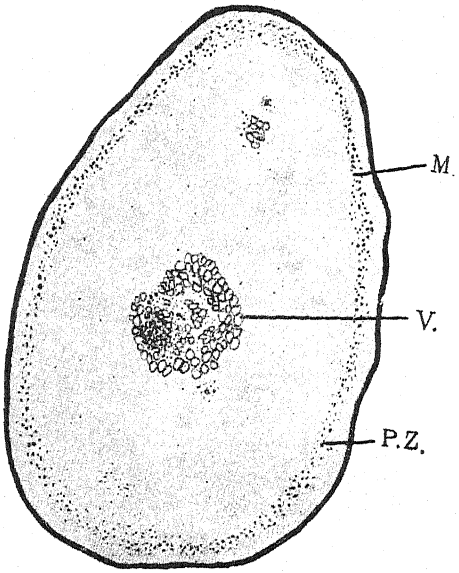

Fig. 6

Fig. 3 shows the peripheral ring (P.R.) of Golgi bodies as also the cortical ring (C.R.) of smaller Golgi bodies. Da Fano preparation, toned.

Fig. 4 is an early oocyte, showing the accumulation of mitochondria in a juxta-nuclear position, prior to the formation of the regular yolk-nucleus of Balbiani. Champy-Kull.

Fig. 5 shows the dispersal stage of mitochondria after the absorption of the idiosome area. Clouds of mitochondria in patches are visible throughout the cytoplasm. Champy-Kull.

Fig. 6 is an advanced stage of oocyte when patches of mitochondria practically disappear; vacuoles are formed in the central region of the egg and a peripheral zone of mitochondria (P.Z.) is established. Champy-Kull.

LETTERING

C. R. Cortical ring of Golgi bodies.
G. B. Golgi bodies.
M. Mitochondria.
M. P. Mitochondrial patch.
P. Z. Peripheral zone of Mitochondria.
P. R. Peripheral ring of Golgi bodies.
V. Vacuoles.
Y. N. Yolk-nucleus of Balbiani.

ON THE CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF PILA GLOBOSA (SWAINSON)

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1. INTRODUCTION

Pila globosa (Swainson) is a common species in North India and is found in tanks, ponds, lakes, streams and rice-fields. So far as we know, the oogenesis of this ampularia from the point of view of cytoplasmic inclusions has not yet been studied. Gatenby in a series of papers contributed to the Quarterly Journal of Microscopical Science (1917—21) has described the cytoplasmic inclusions in the germ cells of a number of molluscs, *e.g.*, *Helix aspersa*, *Limnaea*, *Patella*, etc. Gatenby and Woodger in 1920 discussed in detail the question of relationship between the formation of yolk and the Golgi bodies, mitochondria, etc., during oogenesis in the molluscs and several other types. Other workers in the field have been Hirschler (8) and Weiner (13). Ludford (9) described in detail the oogenesis of Patella in 1921.

The nature and behaviour of cytoplasmic inclusions in *Pila globosa* differ in several marked ways from those described in other molluscs by the abovementioned authors. Thus, there appears to be sufficient justification for adding to our store of knowledge regarding the oogenesis of molluscs from the point of view of cytoplasmic inclusions.

2. MATERIAL AND TECHNIQUE

Large numbers of *Pila globosa* of various sizes were obtained from tanks, round about Allahabad. The ovary was dissected out as quickly as possible and placed in the fixatives, the whole process never taking more than a couple of minutes. The fluids used were Da Fano, Cajal, Kopsch, Ludford's latest modifications of osmic acid methods, Regaud, Regaud-Tupa, Champy-Kull, Flemming without acetic, Zenker-Helly, Dietrich, Kolatchev-Nassonow modifications and some other fixatives which gave us less satisfactory results. Da Fano slides were toned in the usual way. Osmic slides were bleached by Henneguy's method.* These slides were also treated with pure turpentine for varying lengths of time (impure turpentine decolourizes the sections completely in a few seconds); this is also the case with impure xylol or acidic xylol-balsam kept exposed to light for a long time. We would like to lay emphasis on the fact that cold fixation methods gave us very satisfactory results. This method consists in keeping the tubes of fixatives in an ice-box generally for 12 to 24 hours at a temperature of 0°C. This was done particularly with Da Fano, Cajal, Regaud, Regaud-Tupa, and Champy materials. Somehow, this method of fixation gives better results than the method of fixation at ordinary room temperature. We have arrived at this conclusion after trying both the methods side by side.

3. GOLGI BODIES

The Golgi apparatus in young oocytes consists of a number of short batonets or granules arranged in and around a dense area of cytoplasm (Archoplasm) in a juxta-

* Henneguy's method consists in washing slides rapidly before staining, in 1 % aqueous solution of $KMnO_4$ and afterwards in a 4 % solution of oxalic acid. The bleaching should be controlled under the microscope. Sometimes over-osmication is an advantage because in certain cases Golgi bodies take in their characteristic black colour long after the fat bodies have been blackened.

nuclear position (Fig. 1, Y.N.B.). In the absence of a better name, which may be universally accepted, we prefer to call this area by its old name—"the yolk-nucleus of Balbiani." The Golgi elements lie so densely packed together that it is difficult to ascertain their exact nature. At this stage there are visible signs of the formation of fat or fatty yolk. Ludford slides, in a slightly more advanced oocyte, show the formation of fatty yolk to such an extent that the original Golgi batonets are difficult to make out.

In slides treated with turpentine or after Henneguy's bleaching method a certain number of fatty vacuoles disappear, leaving behind still a large number of black spherical bodies. These black bodies of Ludford preparations, which we identify as Golgi bodies, enclosing probably bits of archoplasm, become distributed throughout the cytoplasm in the usual way. A bipolar arrangement of Golgi bodies and the formation of fatty-yolk (Fy.) is well illustrated in Fig. 2. At a later stage, the yolk-nucleus of Balbiani completely disappears and the Golgi bodies are either used up in the formation of the fatty yolk, to be described later or tend to become ultramicroscopic (Fig. 4). In Da Fano preparations, immediately after the disappearance of the archoplasmic area, Golgi bodies emerge as crescent-shaped structures and for a time keep their identity distinct. Here and there the spherical types of Golgi elements also may easily be distinguished (Fig. 3).

4. MITOCHONDRIA

The Mitochondria in the early stages appear to be granular, lying mostly in the region of the juxta-nuclear archoplasmic area (Fig. 5, M.). At a later stage there is sometimes a tendency for the mitochondria to become arranged in a perinuclear layer with a pronounced bipolar arrangement (Fig. 6). From the dense area or idiosome, as it is sometimes called, the mitochondria begin to disperse

like the Golgi bodies and in later stages are more or less evenly distributed throughout the cytoplasm (Fig. 7). In slides fixed by Flemming without acetic acid and stained by iron alum hæmatoxylin the small filamenter type of mitochondria could be distinguished (Fig. 6, FM.) which, however, are not visible in more advanced oocytes. On very careful examination these filaments appear to be formed of granular mitochondria arranged in chain-like formations. This filamenter type of mitochondria was not observed, however, in slides that were prepared by several of the other mitochondrial methods that were employed. Naturally, therefore, we mention the existence of filamenter mitochondria with some diffidence. We would like to add here that the filamenter type of mitochondria was also observed in Unio by the F.W.A. method. One notable fact needs emphasis that in later stages of development so far as could be discerned, the mitochondria become far more numerous than Golgi bodies. The latter are, so to speak, driven out of the field and either become ultramicroscopic or are used up in the formation of fatty yolk. In a well-advanced oocyte the Golgi bodies are not visible at all and the entire egg is full of yolk, nucleolar extrusions, and mitochondria, in various stages of development (Figs. 8, 9).

5. THE YOLK BODIES

Yolk globules of a fatty nature, fairly easily extractable in turpentine, make their appearance at a very early stage in the development of the oocyte. In Ludford preparations they can be distinguished even before the yolk-nucleus of Balbiani is properly established (Fig. 1). When the archoplasmic area is well formed the Golgi bodies are absolutely masked by the formation of an immense number of fatty globules which we propose to call fatty yolk, as suggested by Gatenby and Vishwanath (11). Since these fat globules arise in the area occupied by Golgi bodies and hide the latter from view we conclude that a large number of Golgi

bodies contribute to the formation of these fat globules or fatty yolk (Figs. 2 and 4). The dispersal of Golgi bodies and their accumulation in patches in the cytoplasm (sometimes a bipolar condition is reached as in Fig. 2) are accompanied by the formation of fatty yolk in these areas. A large number of Golgi bodies thus appear to lose their identity as such and become converted into fatty yolk at a comparatively early stage in the development of the oocyte. With the dispersal of Golgi bodies throughout the cytoplasm fatty yolk bodies appear to fill up practically the entire cytoplasm. Comparatively fewer mitochondria and true yolk are visible at this stage. Curiously enough, a fatty yolk sphere when fully formed begins a process of disintegration (Fig. 4). Treatment with turpentine shows that such yolk spheres which have started disintegration become easily decolourised, whereas the others resist the action of turpentine much longer. When the process of disintegration of fatty yolk is at its zenith, Golgi bodies disappear from view and mitochondria become more and more prominent. True yolk formation now begins. So far as we can make out there are two methods of true yolk formation. In one case, the spherical mitochondria swell out into globular bodies by absorption of the necessary materials from the cytoplasm (Figs. 8 and 9, MY.). In the other case (Fig. 9, CY.) a vesicle arises in the cytoplasm and a granule, which stains similarly as the mitochondria, is deposited in the middle of this vesicle. Gradually the granular body swells up and the vesicle also becomes larger and larger. The yolk body thus formed seems to lie in a sort of vacuole. These two kinds of yolk bodies in Ludford slides are grayish black in appearance in sharp contrast with Golgi fatty yolk which goes deep black. Besides, the true yolk or albuminous yolk, as it is called, is not so easily decolourised as the fatty yolk. In a well-advanced oocyte fatty yolk almost completely disappears and its place is taken up by albuminous yolk.

6. DISCUSSION

In the early stages, owing to the formation of the fatty yolk, the exact shape of the Golgi bodies in the yolk-nucleus of Balbiani area is rather difficult to determine in osmic preparations. Da Fano slides reveal their identity as granular bodies in the early stages and as semi-lunar or spherical bodies at a slightly later stage. Figure 3 shows several crescent-shaped bodies with chromophilic rim (CL.) enclosing a spherical chromophobic area (CW.). Distorted forms of various shapes also abound. In other molluscs, Gatenby and Ludford (4, 5 and 9) have described the Golgi bodies to consist of dictyosomes and batonets. There does not seem sufficient reason to doubt that Pila differs markedly from them in this respect. Spherical bodies with black rim are however found, though less frequently, in Ludford, Da Fano and Cajal preparations. According to Parat (12) these spherical bodies would correspond to his vacuome and the dictyosomes to the lepidosome. We have, however, no doubt that these forms are true Golgi bodies and the statement of Parat (7 and 12) that the dictyosomes are modified mitochondria or lepidosomes, as he calls them, so far as this animal is concerned, is difficult to maintain. All the tests that are applicable to the Golgi bodies have been used with success in the case of these dictyosomes.

The mitochondria in the early stages are far less numerous but in an advanced oocyte appear to drive the Golgi bodies out of the field altogether. They certainly multiply in some way or other and become extremely numerous. The growth of mitochondria from a small granule to a fairly large swollen form is easy to follow, as all intermediate stages are found, particularly in Regaud-Tupa preparations (Figs. 8 and 9, MY.). The chief point to note here is the presence of what look like filamenter mitochondria (Fig. 6, FM.) which have not been described yet in any of

the other molluscs. The filaments are short and appear like beaded chains giving one an idea that they are composed of a number of granules. The mitochondria do not form any definite zones such as are met with in the Vertebrates, though in the earlier stages a peri-nuclear zone sometimes appears to exist.

The yolk formation in this animal is not difficult to follow. Ludford slides and most of the other osmic preparations reveal the presence of a large number of fat globules in the earlier stages before the establishment of a definite yolk-nucleus of Balbiani area.

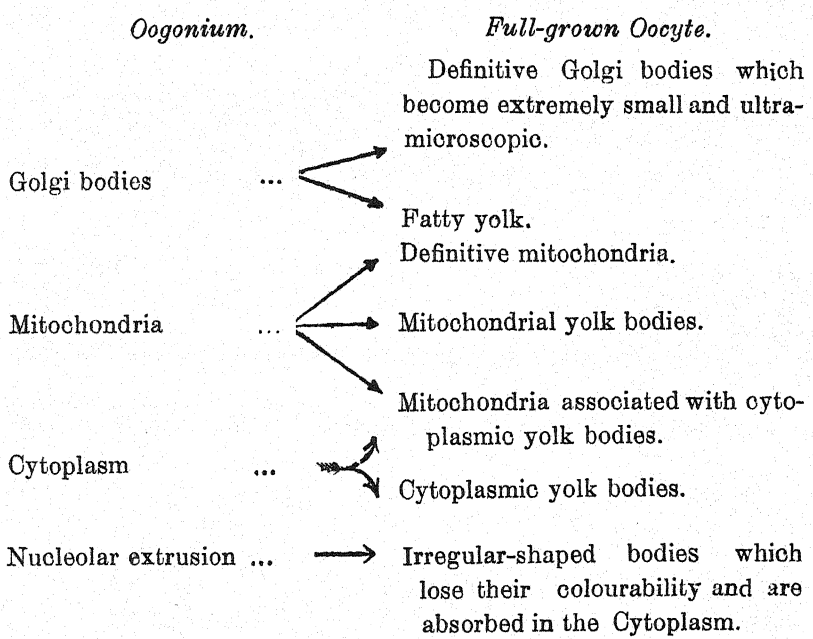
Fewer Golgi bodies apart from these yolk granules are visible in these stages. When the yolk-nucleus area is established the entire mass, as it were, stains black (Fig. 1). On a careful examination under the high powers of the microscope distinct black globules are visible and in between them lie smaller granular bodies which we identify as Golgi bodies. Neither mitochondria nor nucleolar extrusions appear to play an active part in the formation of these fatty yolk globules. So, the latter could only arise either from the cytoplasm or in some direct or indirect way from the Golgi bodies. The fact that these fatty yolk globules appear in the early stages in the yolk-nucleus of Balbiani area and in later stages in patches where Golgi bodies exist (Fig. 2, Fy.), lends colour to the view that they are formed from Golgi bodies. The point that they are not ordinary fat vacuoles and do not arise by sheer coincidence in close proximity to the Golgi bodies is met by the facts that (i) they are not as easily decolourised in turpentine as fat vacuoles generally are, and (ii) when they are decolourised they appear to leave behind a fine black rim. These facts suggest that they are fatty yolk derived either directly or indirectly from Golgi bodies. The fatty yolk formation continues till the end of the dispersal of Golgi bodies throughout the cytoplasm. *Pari passu*, with the secession of the activities of Golgi bodies in

the oocyte, the fatty yolk formation comes to an end. At this stage the egg is chocked—full of fatty yolk. Disruption and disintegration of yolk bodies now begin and in a short time many of the fully formed bodies break up and begin to disappear from view (Fig. 4). Mitochondria now begin to multiply rapidly. Some of the larger and probably older ones become swollen up and assume a size nearly three times as big as an ordinary fully grown mitochondrium. These gradually become converted into yolk spheres and can easily be distinguished from the well-grown fuchsinophil mitochondria, not only by their size but also by the loss of their power of colourability.

Another method of yolk formation seems to exist side by side. Vacuoles appear in the cytoplasm and a granule is deposited inside them (Figs. 8 and 9, CY.). This granule is slightly less fuchsinophil than mitochondria of about the same size. The granule develops inside the vesicle and swells up apparently by the accumulation of materials from the cytoplasm. Instances are also found where a number of small mitochondria arrange themselves on the periphery of the vacuoles (Fig. 9). It is possible that the granular mitochondria help indirectly in the formation of the cytoplasmic yolk sphere in much the same way as the Golgi elements do in the formation of the yolk spheres in Patella (6 and 9).

Nucleolar extrusions abound (Fig. 10) but do not appear to play any important part in the formation of yolk. They are irregular bodies and soon lose their power of colourability and are lost in the general cytoplasm.

The fate of the cytoplasmic inclusions in this Ampullaria may be graphically represented thus :—


Oogonium.
Full-grown Oocyte.
Definitive Golgi bodies which become extremely small and ultra-microscopic.
Golgi bodies ...
Fatty yolk.
Definitive mitochondria.
Mitochondria ...
Mitochondrial yolk bodies.
Mitochondria associated with cyto-plasmic yolk bodies.
Cytoplasm ...
Cytoplasmic yolk bodies.
Nucleolar extrusion ...
Irregular-shaped bodies which lose their colourability and are absorbed in the Cytoplasm.

7. SUMMARY

1. The Golgi elements consist mostly of granules, dictyosomes or batonets. More rarely spherical Golgi bodies are also found.

2. Golgi bodies at a very early stage contribute directly or indirectly to the formation of fatty yolk spheres.

3. Most of this fatty yolk disintegrates and is absorbed in the cytoplasm at a stage when true yolk formation (albuminous yolk) begins.

4. At this stage mitochondria multiply rapidly, swell up and give rise to true yolk spheres by a process of direct metamorphosis.

5. At an early stage of development the filamenter type of mitochondria could be observed in material fixed by the F. W. A. method.

6. The cytoplasm also appears to give rise to yolk spheres by the formation of vesicles inside which granules appear. Mitochondria in many cases become stuck on the periphery of the vacuole and may play some indirect part in the formation of this type of yolk sphere. It is also possible that the granules noticed inside the vesicle are developing mitochondria.

7. In a fully formed Oocyte, mitochondrial and cytoplasmic yolk spheres abound, though here and there some fatty yolk spheres (Golgi) may also be found.

8. Nucleolar extrusions do not appear to take any part in the formation of yolk bodies.

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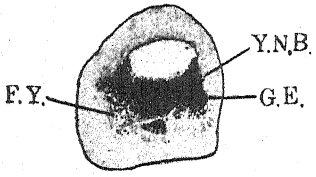
PLATE 1



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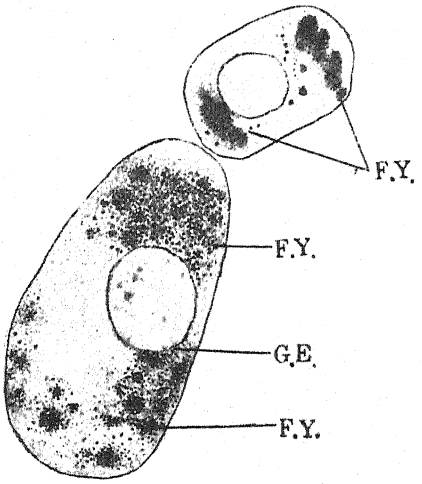

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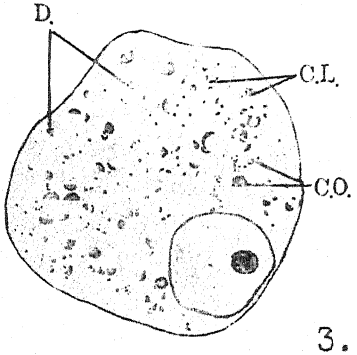

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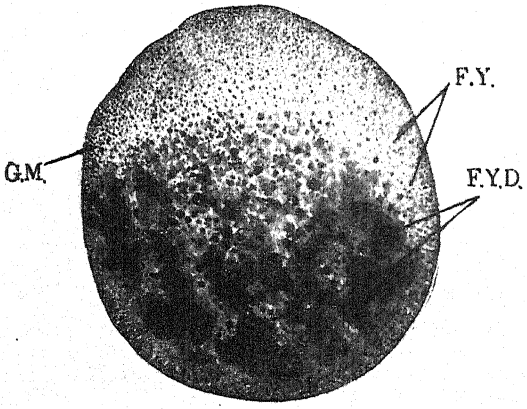

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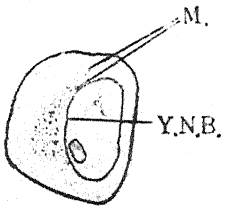

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EXPLANATION OF PLATES

Fig. 1. A camera lucida sketch of an oocyte showing granular Golgi bodies in the archoplasmic area (yolk-nucleus of Balbiani); fatty yolk formation has started ; Ludford preparation.

Fig. 2. A camera lucida sketch of a Ludford slide showing bipolar arrangements of Golgi bodies and fatty yolk formation.

Fig. 3. A camera lucida sketch of a Da Fano slide showing dictyosomes.

Fig. 4. A Ludford slide showing fatty yolk formation and disintegration.

Fig. 5. A camera lucida sketch of F. W. A. showing the mitochondria in the region of archpolasmic area.

Fig. 6. A camera lucida sketch of F.W.A. slide showing a perinuclear ring of granular mitochondria, and also the filamenter type of mitochondria.

Fig. 7. Champy preparation showing the archoplasmic area and the conversion of mitochondria into yolk.

Fig. 8. Regaud-Tupa preparation showing the direct transformation of mitochondria into yolk.

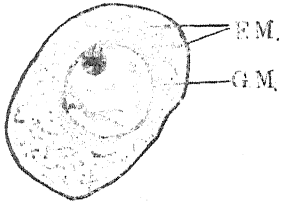
Fig. 9. A camera lucida sketch showing mitochondrial and the cytoplasmic yolk in various stages of development. Flemming-Champy-Kolatchev preparation.

Fig. 10. Bouin preparation showing nucleolar extrusions.

EXPLANATION OF LETTERING

CL. Chromophilic ring,
CO. Chromophobic substance,
CY. Cytoplasmic yolk,
D. Dictyosomes,
FM. Filamenter type of mitochondria,
Fy. Fatty yolk,
G.E. Golgi elements,
G. M. Granular mitochondria,
MY. Mitochondrial yolk,
NE. Nucleolar extrusions,
Y.N.B. Yolk-nucleus of Balbiani (Archoplasm),
F.Y.D. Fatty Yolk disintegration.

PLATE 2



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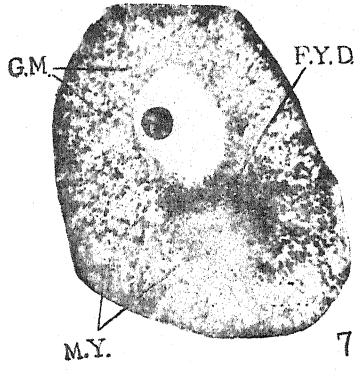

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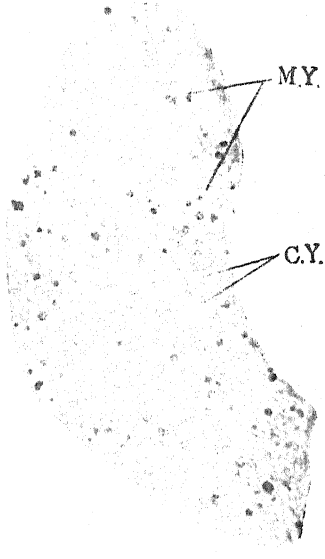

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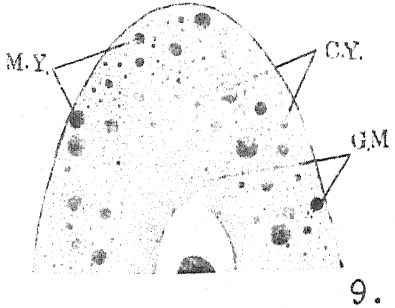

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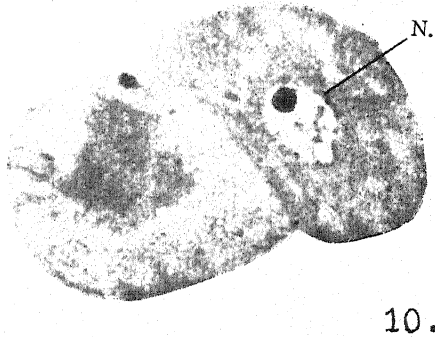

10.

CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF BUFO MELANOSTICUS, RANA TIGRINA AND RHACOPHORUS FERGUSONII

BY

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CONTENTS

INTRODUCTION

In 1906 Lams (20) carried out some work on the formation of the Vitellus of the Amphibian egg. Lams, however, did not employ the methods now used for the detection of the Golgi bodies, neither did he undertake seriously the question of the metamorphosis of the mitochondria into yolk. Gatenby (12) in his work on Amphibian Oogenesis remarks: "It seems natural to conclude that the yolk-grains of the egg are formed from the metamorphosed mitochondria, as is believed to occur in other animals. It should be mentioned that in both insects and Amphibians the mitochondrial elements become so fine that it is difficult to tell whether the yolk is being formed from them or not. In preparations of frog ovary by the Mann-Kopsch method the mitochondria of the oocyte impregnate in a different manner from the yolk, and no certain transitional forms can be noted. We consider that the matter is not settled."

The question of mitochondria in the oogenesis of Amphibia has also been treated by Schmidt (23) and Jorgenson

(16) in triton, and in frog by Konopacki (17). But none except Hibbard (15) seems to have tried so far to make a comprehensive study of all the cytoplasmic inclusions from the point of view of the modern cytology. In order to fill up this gap Dr. Bhattacharya suggested to me to undertake this work. I am submitting in this paper my observations on three species of Amphibia, but I hope to continue the work on a number of other species, and present later on a comparative account of all the cytoplasmic inclusions in the Amphibian oogenesis.

This work has been carried on under the guidance and supervision of Dr. D. R. Bhattacharya. I wish to express my deep sense of gratitude for the keen interest which he showed throughout the progress of this work, and for kindly correcting the MS. of this paper.

MATERIAL AND TECHNIQUE

Toads (Bufo melanosticus), and frogs (Rana tigrina) abound at Allahabad and can be obtained at any time of the year. Rhacophorus fergusonii, however, is difficult to obtain as I came across only two specimens in this locality during the last two years. Through the kind efforts of Dr. A. B. Misra of Benares University I was able to obtain a dozen live specimens from Nepal-Tarai. The ovaries were taken out as quickly as possible to avoid the chances of post-mortem changes. The following methods of fixation were used in connection with this work :—

1. Da Fano's Cobalt nitrate modification : cold fixation.
2. Roman Y Cajal's Uranium nitrate and silver nitrate method : cold fixation.
3. Latest modification of Mann-Kopsch Osmic method by Ludford (19).
4. Flemming without acetic acid.
5. Regaud's Formol-bichromate method.

6. Regaud-Tupa's Uranium nitrate formol-bichromate method.
7. Bouin's Picro-formol method.
8. Champy's method modified by Nassonov and later by Parat: cold fixation.
9. Hermann's method.
10. Carnoy's method.
11. Helly's method.

The cold method as suggested by Parat (22) and Bhattacharya (3) consists in placing the material in the fixative for the necessary period at 0°C in an ice-box. By comparing the results with the material thus fixed, with that fixed at ordinary room temperature, it is found that the cold fixative methods give by far the best results. The shrinkage is comparatively much smaller and the fixative remains good at this temperature for a much longer time than otherwise, and therefore its action on the tissue is more of the desired nature.

The ovaries of toad and Rhacophorus are more osmophil than those of frog and it is inadvisable to leave them in 2% osmic acid for more than three days ; but frog's ovaries can be safely kept on for about a week. In summer the time had to be shortened to about 36 hours. If kept longer the material gets brittle and has a tendency to disintegrate, and also requires plenty of bleaching. Osmic acid at room temperature works well, but if kept in a hot bath as recommended by Ludford Champy and others, the acid as well as the material soon gets spoilt, becoming disintegrated and powdery. I find that sections from osmic preparations should always be treated with turpentine for 20—30 minutes in order to dissolve away the fat. The turpentine must invariably be of the purest variety and not acidic. Similarly sections should be mounted in pure Canada-balsam (Baume du Canada). Ordinary balsam exposed to light soon becomes

acidic, and sections mounted in this medium lose their colour and get spoilt in a short time.

For the purposes of intra-vitam examination very young ova were thoroughly teased out in salt solution (6/1000) dilution and examined in neutral red solution (1/500 in 6/1000 salt solution).

Ovaries were also teased out in salt solution and examined after the addition of a few drops of 2% Osmic acid according to the method recommended by Gatenby and Nath (14).

DESCRIPTION

Golgi Bodies.—The earliest stages of the Golgi apparatus can be detected only with great difficulty in very young oocytes (Fig. 1). It lies close to the nucleus in the form of a minute knot-like body. The constitution of this compact little mass is hard to determine even under high powers of the microscope. As the oocyte develops, this mass begins to spread itself out in a semi-lunar fashion. In still bigger oocytes the apparatus forms a little cap-like structure on one pole of the nucleus. In some oocytes more than one such caps are noticeable. In later stages the Golgi bodies begin to spread themselves out around the nucleus and occupy a perinuclear position (Fig. 2), but they never completely surround the nucleus in such a way as to form a regular perinuclear zone. After this process of distribution is completed the Golgi bodies appear to undergo disruption and resolve themselves into more and more minute granule-like structures. The Golgi elements thus become extremely numerous at this stage. They have now a tendency to arrange themselves in a ring-like manner more or less midway between the nucleus and the extreme periphery of the egg—a feature which is characteristic of tortoises (2) and fowls (6) and many other vertebrates. From this position the Golgi bodies move towards the periphery (Fig. 3) as yolk

formation proceeds. This general account of the behaviour of the Golgi bodies is common to all the three types I have examined—Bufo melanosticus, Rana tigrina, and Rhacophorus fergusonii. Mention might be made here of the presence of a layer of fine dust-like particles in the extreme periphery of the egg (Figs. 4 and 7). I have reason to believe that these are Golgi elements derived from the follicular epithelium of the egg.

It is remarkable that the Golgi bodies after emerging from the juxtanuclear mass always appear as minute spherical bodies and are never irregular in shape if properly fixed.

By the intra-vitam examination method (3) with neutral red, very young oocytes showed a juxtanuclear patch of minute spherical bodies which take in the characteristic red colour.

In older oocytes the dispersed condition of Golgi bodies could be readily noticed. According to Parat (22) these should be termed Vacuome and should be regarded homologous with the Golgi bodies as demonstrated by the classical methods. At this stage of our knowledge, however, I feel disinclined to go into the controversy of the Golgi bodies and the Vacuome theory.

Migration of Golgi bodies.—In silver nitrate preparations after proper toning and in osmic preparations after bleaching, it is possible to demonstrate the Golgi apparatus in the follicular epithelium. In the earliest stages observable they appear as a dense granular mass between the nucleus and the zona radiata. This mass later on gives rise to a considerable number of small granular bodies which react to the silver and osmic fixatives in the same way as the Golgi bodies do inside the eggs. These bodies travel through the zona radiata and become lodged in the extreme periphery of the egg. Thus stages showing the migration of the Golgi bodies from the follicular epithelium

to the zona radiata and from the zona radiata to the egg could be distinctly seen—particularly in the frog and Rhacophorus (Figs. 4 and 5). This migration of Golgi bodies has already been described by Bhattacharya (2) in tortoise and other animals (4) and by Brambell (6) in fowl.

Mitochondria.—The mitochondria can be demonstrated beautifully by Regaud-Tupa and Champy methods. They make their first appearance as a dense cloud in the usual juxta-nuclear position (Fig. 6). Later, in a fairly grown-up oocyte the mass breaks up into very fine granules and disperses throughout the cytoplasm (Figs. 8 and 9). I have found only the granular type of mitochondria in all the three animals I have examined. Konopacki (17) has found the filamenter type of mitochondria in addition to those of the granular type in the frog with Regaud's long method as described by Bulliard (7). I examined slides after two to six months' fixation, but did not succeed in getting the filamenter type of mitochondria.

Nucleolar Extrusions.—These could be seen very nicely in material fixed in Bouin, Carnoy and Helly's fixatives. In very young oocytes the nucleus contained a single nucleolus, but in older oocytes a number of big rounded bodies could be seen, sometimes as many as five. The original nucleolus disappears at a very early stage and at a later period fragments spherical in shape make their appearance inside the nucleus and arrange themselves more or less regularly near the nuclear membrane (Fig. 10). These are extruded into the cytoplasm on all sides of the nucleus. These emissions are fairly big in the frog and Rhacophorus, but are fine and granular in toad. They lose their power of colourability and are soon lost in the general cytoplasm. The nuclear extrusions do not seem to take any direct part in yolk-formation.

Yolk.—Fatty yolk-formation starts at a very early stage in the development of the oocytes of these animals. It is an

established fact when the juxta-nuclear mass appears. In Ludford preparations fatty yolk bodies appear to lie side by side with the Golgi bodies. These yolk discs take an intense black colour with osmic acid indicating that they are formed of fatty yolk. The minute Golgi bodies hold the black colour more tenaciously than the swollen up ones. In the latter turpentine can remove the blackness more easily from the central area—the outer ring retaining the black colour much longer. This shows that the swelling is due to the accumulation of fat inside the vacuole. In young oocytes the entire cytoplasmic area appears full of black particles, which are Golgi bodies just beginning to swell up. Later, when these have swollen further and formed fatty yolk discs they arrange themselves in the cortical region in a ring-like zone—the medullary region being more or less free from fatty yolk in the older oocytes. This layer gradually travels outwards with the growth of the oocytes. The Golgi bodies thus appear to take part in the formation of yolk; although, I am not sure if they do so by direct metamorphoses.

The true yolk appears to be derived from the mitochondria. In Champy and Hermann preparations a gradual swelling of mitochondria may be noticed. The mitochondria can be shown in osmic preparations by Champy-Kull staining, and it is found that they are swelling up and forming small yolk discs side by side with the Golgi bodies which form bigger discs of fatty yolk (Fig. 11). The process of the conversion of mitochondria into yolk discs starts near the periphery and gradually travels towards the medullary region. This confirms Gatenby's observations on the behaviour of mitochondria in the frog (12).

SUMMARY

The Golgi bodies are noticed in very young oocytes in the usual juxtanuclear position at one pole of the nucleus. Later they begin to spread out and take a perinuclear position from where they disperse in the general cytoplasm, and form fatty yolk.

In older oocytes a definite layer of dust-like Golgi bodies is found in the cortical region near the zona radiata. There is evidence to believe that these bodies migrate from the follicular epithelium to the egg.

In the early stages of the development of an oocyte the mitochondria form a cap-like dense cloud over one pole of the nucleus. Later they disperse and become distributed throughout the cytoplasm. Only spherical mitochondria could be detected.

True yolk is formed by the metamorphoses of mitochondria. Neither nucleus nor cytoplasm appears to take a direct part in the formation of true yolk bodies.

Very minute spherical fragments are extruded from the nucleus on all sides. They lose their power of colourability and are lost in the general cytoplasm.

EXPLANATION OF FIGURES

Figures 6, 7 and 9 have been drawn under Lietz's Camera Lucida and the rest are microphotographs. The objectives and eye-pieces used are all Lietz's make except the oil immersion lens which is Zeis' make.

Figure 1. Young oocytes of Rana tigrina showing juxta-nuclear position of Golgi apparatus (G1)—oil immersion 1/12. E. P. 4x.—Ludford.

Figure 2. Oocyte of Bufo melanosticus showing perinuclear position of Golgi apparatus (G1)—oil immersion 1/12. E.P. 4x.—Cajal.

Figure 3. Oocyte of Bufo melanosticus showing Golgi bodies (G1) dispersing out and travelling towards the periphery of the egg. Obj. 8. E.P. 3x.

PLATE I

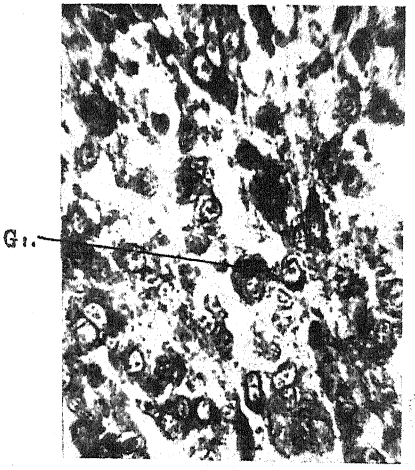


Fig. 1.

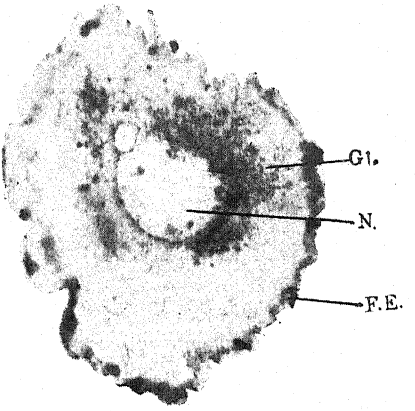


Fig. 2.

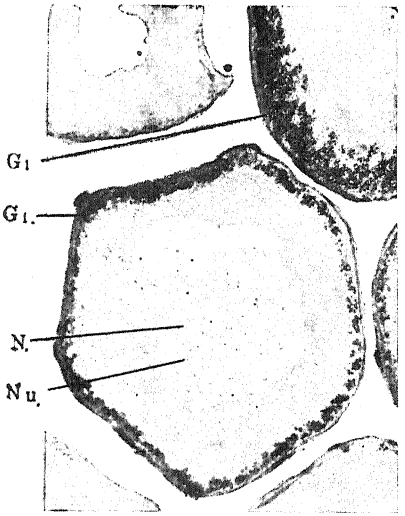
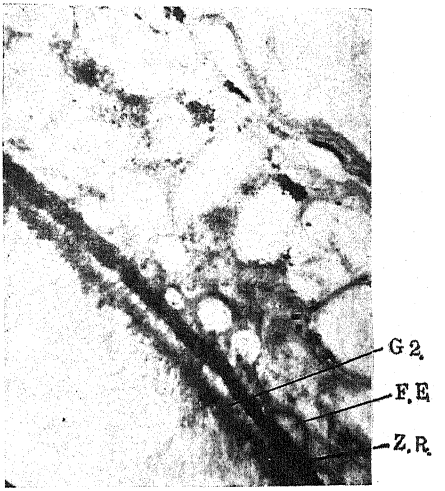


Fig. 3.



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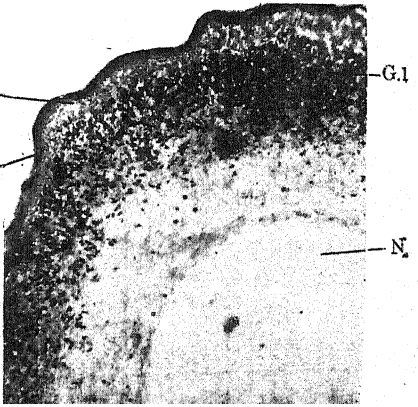

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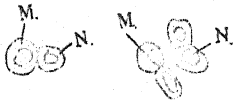

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Fig. 6.

0·1 MM

Fig. 7.

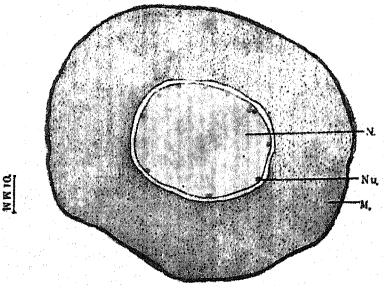


Fig. 8.

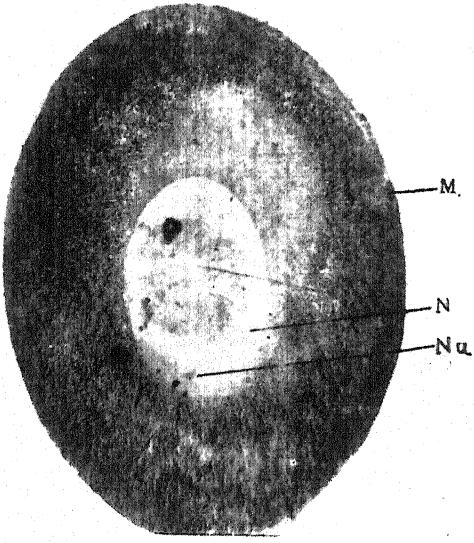
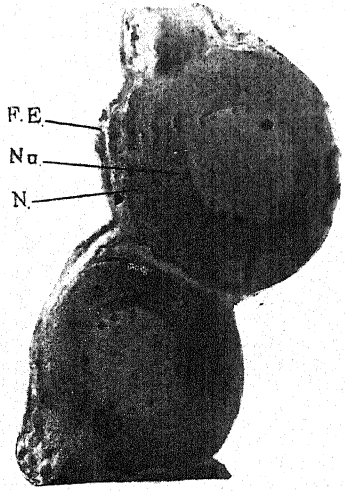


Fig. 9.



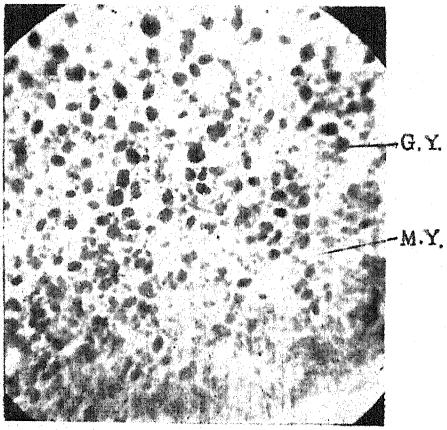


Figure 4. Part of an old oocyte of Rana tigrina showing minute Golgi bodies (G2) filtering from the follicular epithelium (F. E.) through the zona radiata (Z. R.) into the oocyte—1/12 oil immersion. E.P. 4x.—Da Fano.

Figure 5. Oocyte of Rhacophorus fergusonii showing Golgi bodies (G1) arranged in a ring and travelling towards the periphery. Internal to the zona radiata is seen a layer of very minute dust-like Golgi bodies (G2) which have filtered in from the follicular epithelium (F. E.). Obj. 8. E.P. 4x.—Da Fano.

Figure 6. Young oocytes of Bufo melanosticus showing the juxta-nuclear patch of mitochondria (M)—1/12 oil immersion. E.P. 4x.

Figure 7. Oocyte of Bufo melanosticus showing perinuclear patch of mitochondria (M)—1/12 oil immersion. E.P. 4x—Regaud-Tupa.

Figure 8. Oocyte of Bufo melanosticus showing mitochondria (M) dispersed out in the general cytoplasm. Obj. 8. E.P. 4x.—Hermann.

Figure 9. Old oocyte of Rana tigrina showing mitochondria (M) dispersed out in the general cytoplasm—1/12 oil immersion. E.P. 3x.

Figure 10. Oocytes of R. tigrina showing numerous nucleoli (Nu). Obj. 8. E. P. 4x.—Bouin.

Figure 11. Part of a mature egg of Bufo melanosticus showing mitochondrial (M. Y.) and Golgi yolk (G. Y.) discs. 1/12 oil immersion. E. P. 4x.—Ludford.

LETTERING

A—Archoplasm.
F. E.—Follicular epithelium.
G1—Golgi bodies belonging to the oocyte.
G2—Golgi bodies coming into the egg from the follicular epithelium.
G. Y.—Golgi yolk disc.
M—Mitochondria.
M.Y.—Mitochondrial yolk disc.
N—Nucleus.
Nu—Nucleolus.
Z.R.—Zona radiata.

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THE INFILTRATION OF GOLGI BODIES FROM THE FOLLICULAR EPITHELIUM TO THE EGG IN FISHES

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1. INTRODUCTION

In the year 1924, Brambell (3) in the fowl and Bhattacharya (1) in the tortoise first brought to light the interesting fact that the egg during the course of development receives a sufficient quota of Golgi bodies from the follicular epithelium. It appears that like the process of nucleolar extrusions, there are two ways in which Golgi bodies are extruded from the follicular epithelium to the egg. In the first case, e.g., in the fowl (3) the Golgi bodies are extruded in fairly big lumps and can be seen in the zona radiata and in the cortical region of the egg in all stages of descent. In the second case, e.g., in the tortoise (1), the Golgi elements are extremely minute and globular bodies and are found to filter through, as it were, in beaded chain-like formations via the fine striated passages of the zona radiata to the egg, which is well differentiated at a particular stage of development. In a paper (2) just published, Bhattacharya in collaboration with Das and Dutta has shown the infiltration of Golgi bodies from the egg membranes to the egg in certain Amphibians, Reptiles and Birds.

The Vertebrate groups of Fishes and Mammals remain still untouched. It is with a view to fill up this gap that the present work was suggested to me by Dr. Bhattacharya.

I have pleasure in acknowledging here my sincere thanks to Dr. Bhattacharya, under whose guidance the work was carried out. I am also indebted to Mr. S. K. Dutta who helped me in various ways.

2. OBSERVATIONS

The fishes examined were Saccobranchis fossilis and Ophiocephalus punctatus. The following observations are based mostly on the latter. The technique employed was mostly Da Fano's Cobalt nitrate method. Cajal's Uranium nitrate method and Ludford's latest modification of the osmic method were also tried, but they gave less satisfactory results. Sections 5-6 M thick were cut and toned in the usual way. The fixation was done in the cold at 0°C.

Fig. 1 is a semi-diagrammatic sketch of a portion of the egg of Ophiocephalus punctatus. The egg is in a fairly advanced stage of development. The zona radiata is well established and infiltration (Inf) of Golgi bodies is visible even under the low power of the microscope.

The process of infiltration appears to begin at a pretty early stage in the development of the egg, even before the zona radiata makes its appearance as a distinct layer. It decreases in intensity as the egg grows till at the stage depicted in Fig. 1, we find the process of infiltration is confined to a number of patches, of which three are shown in the figure. In this fish the follicular epithelium is represented as a very thin area and appears to be double-layered. Da Fano's fixation does not fix the epithelium so well as Cajal does.

Fig. 2 is a semi-diagrammatic sketch of a portion of the egg drawn in Fig. 1, under high power. The follicular epithelium is more distinct here, though the cell boundaries

are not visible. There is always a larger accumulation of fine dust-like particles of Golgi bodies near the nucleus and the zona radiata. Prior to their extrusions they seem to mass together on the border adjacent to the zona radiata layer or the investing egg membrane, when the zona is not yet well established. This stage is preparatory to the next when the Golgi elements are extruded from the follicular epithelium to the egg. At this stage both fine dust-like particles of Golgi elements as well as big irregular-shaped lumps of Golgi bodies are visible in the zona radiata.

All stages of descent from the epithelium to the egg via the zona radiata can easily be made out under an oil immersion lens. After reaching the egg, the Golgi bodies settle down in the cortical region of the egg and form a dense layer of granular bodies. The granular Golgi elements are the rule, though here and there, larger lumps of Golgi bodies may be seen. Whether these lumps are the result of Golgi granules running together under the influence of fixatives or are the result of growth of certain Golgi bodies, I am unable to tell. These loose lumps even in slides thoroughly well washed and toned give rather an idea that the former alternative is more probable.

The thickness of the zona radiata and even the appearance of the striations depend on the stage of development of the egg. Although my sections show eggs in varying stages of development, I have not been able to trace out the finer divisions of the zona radiata as described in certain fishes by Champy and Gley (4). Nor, have I been able to see the canalicular passages as described by Bhattacharya in the tortoise (1 and 2).

Fig. 3 shows two eggs lying side by side, in which the process of infiltration is becoming exhausted. The Oocytes are fairly advanced and larger. Golgi bodies seem to have broken down into smaller ones and started

disappearing from view. With the growth of oocytes, the zona radiata becomes thinner and more opaque.

Fig. 4 shows a Camera lucida sketch of a more advanced egg, under high power. The zona radiata is very thin and the process of infiltration (Inf) is very rare. There is hardly any difference now between the Golgi bodies (G.B.) that come from the follicular epithelium and those (G.B.) that were originally formed in the egg. Both the types have a tendency to break up and become ultramicroscopic.

3. CONCLUSION

In the fishes examined neither the fibrillar layer described by Gatenby (5) and Bhattacharya (1), nor the dark and light layers described by Champy and Gley (4) could be found. It is possible that the fixatives I used for the demonstration of the infiltration of Golgi bodies were not quite suitable for the demonstration of egg membranes. Follicular activity is great in the early stages of the development of the oocyte. It is at this period that the infiltration of the Golgi bodies reaches its maximum. The Golgi elements of the epithelium pass into the egg via the zona radiata, either in big lumps or as is more frequent, in the form of granular bodies. In an advanced oocyte, when the yolk formation has fully set in, the extrusion of Golgi bodies becomes much less intense. They can still be observed as lying in patches in the zona radiata, here and there. In a still more advanced oocyte, the process practically ceases and only occasionally a certain patch of Golgi elements in the zona radiata can be distinguished. The zona radiata itself becomes thinner and more opaque.

The Golgi bodies (G.B.) thus derived from the follicular epithelium settle down at the periphery of the egg and form a dense cortical layer. They seem to get mixed up gradually with the original layer of Golgi bodies (G.B.) which at an earlier stage form a peripheral ring internal to the cortical

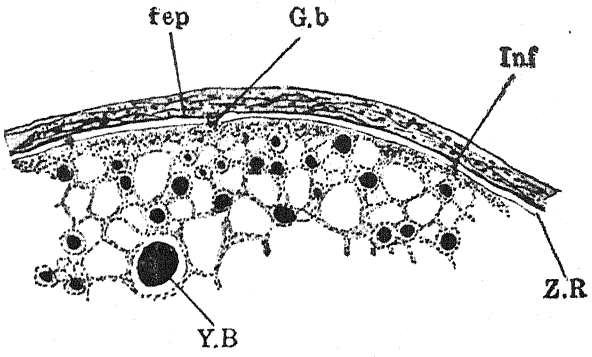

Fig. 1. A semi-diagrammatic sketch of a portion of the egg of Ophiocephalus punctatus. X 500. Da Fano preparation (toned).

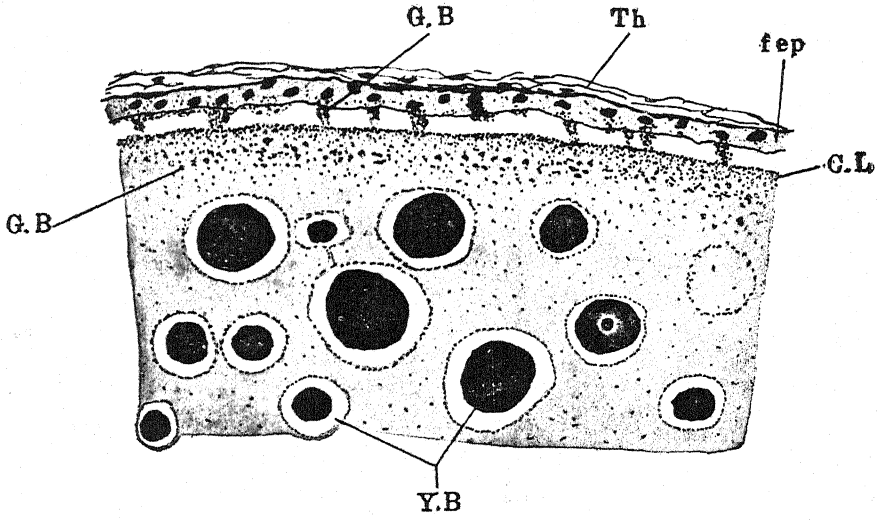

Fig. 2. A semi-diagrammatic sketch of a portion of the egg of Ophiocephalus punctatus under high power. X 1200. Da Fano preparation (toned).

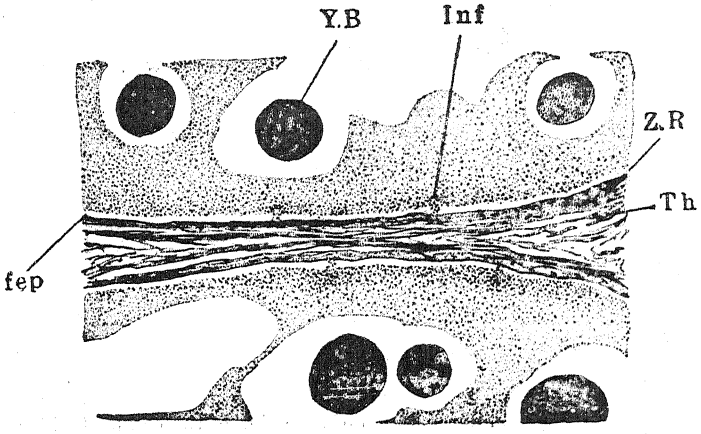

Fig. 3. A sketch under Camera lucida (X 900) of two eggs of Ophiocephalus punctatus lying side by side. Da Fano preparation (toned).

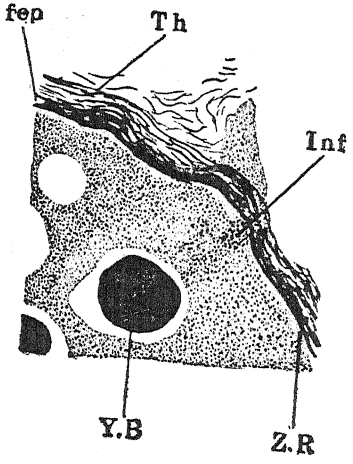

Fig. 4. A Camera lucida sketch (X 1200) of an egg of Ophiocephalus punctatus. Da Fano preparation (toned).

layer of Golgi bodies of the egg. In an advanced oocyte such as is depicted in figures 3 and 4 there is absolutely no way of distinguishing between these two layers. The Golgi bodies appear to break up and become finer, and finer, before being completely lost to view. What part these Golgi elements derived from the follicular epithelium play in the development of the egg, it is difficult to tell. With the development of microscopical technique and specific methods, it may be possible to fix separately the Golgi bodies in the egg derived from the two sources described above. Then it may be possible to follow separately their ultimate fate.

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5. LETTERING

C. L. : Cortical region of the egg full of Golgi bodies.
f. ep.: Follicular epithelium.
G. B. : Golgi bodies of oocyte.
G. b. : Golgi bodies derived from follicular epithelium.
Inf. : Infiltration of Golgi bodies from the follicular epithelium to the egg.
th. : Theca.
Y.B. : Yolk.
Z. R. : Zona radiata.

A NEW NEMATODE PROCAMALLANUS MEHRII, N. SP. FROM A LOCAL SILUROID FISH—WALLAGO ATTU

BY

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The Nematode described in this paper was obtained from the posterior part of the body cavity of *Wallago attu*. It seems to be very rare as out of about forty fishes dissected only four were found infected with it. Two male and one female specimens were found in one, two from another, and one from a third fish. One specimen, a male was obtained from the gas-bladder of one of these fishes. They were examined first in the living condition and subsequently fixed for permanent mounts according to the method devised by Looss (3). The worms as will be seen from the following description belong to a new species of *Procamallanus*—the only species of this genus hitherto known being *P. laeviconchus* Wedl, 1862, *P. spiralis* Baylis, 1923, and *P. parasiluri* Fujita, 1927, from the Siluroid fishes *Synodontis schaal*, *Heterobranchus anguillaris* and *parasilurus asotus* respectively.

I have great pleasure in thanking Dr. H. R. Mehra of the Department for his valuable help and guidance in the preparation of this paper, and Dr. D. R. Bhattacharya, Head of the Department, for various facilities in connection therewith.

The worm is of a fairly great length. It is distinctly divided into three regions, the head, body and tail. The anterior end is flat and broad in both sexes, while the

posterior end is straight and pointed in female (Fig. 4) and sharply curved in male (Fig. 3). The caudal alae, preanal, adanal, and postanal papillae, as usual, are present in the male specimens only. The cuticle covering the body-wall is more or less thickened in the form of a longitudinal ridge, one on each side, and is transversely striated. The excretory pore is situated in the anterior portion of the body and the anal opening lies ventrally at the posterior end in both the sexes. The female opening lies in front of the posterior third portion of the body.

Head.—The buccal capsule has a continuous chitinous wall which does not show any separation into two lateral valves. Tridents are absent, but in their place two knob-like chitinous projections (Figs. 1 and 2) are present ; they arise one on each side from the hinder wall of the floor of the buccal cavity, and are directed backwards and inwards converging more or less towards each other. Their thickness does not exceed that of the chitinous wall from which they arise. As will be seen from the figures they differ remarkably in form, size, and position from the tridents known in the other species of the genus *Procamallanus*. At the anterior end of the buccal cavity there are present four chitinous plate-like structures, of which the two median ones are much larger. They are slightly separated from each other and are covered on the sides by the leaf crowns which are special outgrowths of the body-wall at the anterior end in the head region. The leaf crowns in the neighbourhood of middle plates are 9-10 in number in the male and 12 in the female, whereas those lying close to the outer plates are 6-7 in the male and 10 in the female. Their form also differs in the two sexes, in the male specimen they are more or less conical, while in the female they are elongated and more or less spindle-shaped. In the male the buccal cavity has a diameter of about 1/100th part of the entire body length. In the female, though its length is nearly the same, its

breadth is about 1/160th of the body length, showing thereby that the buccal cavity here is elongated and narrow from side to side. The œsophagus is nearly 1/9th of the total length of the animal. It is divided into two parts, the anterior muscular and the posterior glandular, of nearly equal size. The intestine is nearly a straight tube and opens ventrally at the anus which lies ·102 mm. in front of the hinder end. The nerve ring is situated much anteriorly at about the commencement of the muscular portion of the œsophagus. The excretory system consists of two longitudinal canals, one on each side, which open anteriorly by a separate opening situated laterally about ·7 mm. behind the anterior end in the male and ·95 mm. in the female.

The length of the male specimen is nearly half that of the female one. The testes are tubular and are situated in the anterior portion of the body. The vas deferens of both the sides join to form a common duct which opens to the exterior through the cloacal opening. At the junction of the common vas deferens and the cloaca there are present a pair of chitinoid rods—the spicules which have an elongated conical form being broader at the anterior end and narrower and somewhat tapering towards the caudal end. They are equal in size measuring ·255 mm. in length and ·017 mm. in greatest breadth. A fin fold is present around the tail in the male. Its length varies from ·94 mm. to 1·2 mm. and its greatest breadth from ·17 mm. to ·2 mm. It is a thin cuticular expansion of the cuticle of the body-wall containing several pairs of rib-like papillæ at various places. These papillæ are given names according to their position, *i.e.*, preanal, adanal and postanal. They differ in size, the preanal ones being the largest and the postanal the smallest. They are slightly curved, assuming to a certain extent the form of the letter "S" and more or less resemble in form the setae of an earthworm (Fig. 3). Their ends are, however, somewhat

swollen and knob-like. These papillæ are twenty-three in number and are arranged in eleven pairs, the last unpaired one is situated at the extreme hinder end of the body.

In female the ovaries are elongated tubes which commence near the œsophagus. After reaching the posterior end they turn forwards to join and form a common duct the vagina which opens at the vulva. The latter is situated at a distance of 10·0 mm. from the posterior end in a specimen of 33·0 mm. length. The area of the body-wall around the vaginal opening (vulva) is thickened and swollen ; it is produced into distinct lobes, the anteriormost of which lying just in front of the vulva is the largest and crenated. The others gradually decrease in size and distinctness from before backwards. The lobe on the side opposite to that of the vulva is the largest and lies somewhat anteriorly to it and the crenated lobe in front (Fig. 5).

Table Showing Measurements

	Slide No. 1.		Slide No. 2.		Slide No. 3
	Sp. No. 1.	Sp. No. 2.	Sp. No. 1.	Sp. No. 2.	Female.
1. Total length of the specimen	17·mm	16·	19·	18·	33·
2. Greatest thickness (middle of the body) ...	·323	·34	·34	·357	·425
3. Length of the Buccal Cavity	·17	·17	·17	·17	·35
4. Diameter of the Buccal Cavity	·17	·17	·17	·17	·20
5. Length of the Muscular portion of the œsophagus...	·92	·92	·925	1·14	1·61
6. Breadth „ „ „ „ „ ...	·136	·136	·153	·187	·20
7. Length of the Glandular portion of the œsophagus	·97	·8	·97	1·02	1·41
8. Breadth „ „ „ „ „	·136	·136	·136	·17	·17
9. Length of the body in front of the nerve ring ...	·272	·272	·30	·323	·527
10. „ „ „ „ „ excretory pore ...	·50	·50	·476	·476	·956
11. Interval between two striations in the cuticle ...	·0036	·0036	·0036	·0036	·0036
12. Length of the tail in male (behind the anus) ...	·102	·102	·102	·102	...
13. Length of spicules	·255	·255	·255	·255	...
14. Greatest breadth of the spicules	·017	·017	·017	·017	...
15. Length of the fin fold in the male ...	1·10	·94	1·2	1·1	...
16. Greatest breadth of the fin fold in the male ...	·17	·187	·20	·187	...
17. Length of the tail in the female	...	...	...	...	·23
18. Position of the female opening from the posterior end ...	...	...	...	...	10·0

The Distinguishing Features of the Species are:

1. The presence of a large number of leaf crowns.
2. The absence of tridents and presence in their place of knob-like chitinous structures in the buccal cavity.
3. A well-developed crenated lobe lies in front of the vulva.
4. The "S-" shaped form of the anal papillæ in the fin fold and their number.

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EXPLANATION OF PLATE AND FIGURES

A, Anus. A. L., Anterior biggest lobe opposite to Vulva. Buc. Cav., Buccal Cavity. C. A., Caudal Alæ. C. L., Crenated lobe. C. W., Chitinous wall. G. A., Genital Aperture. Int., Intestine. K., Knob-like structure. L. C., Leaf Crowns. Oeso., Oesophagus. P. L., Posterior lobes. P. P., "S" shaped paired papillæ. Sp., Spicules, St. Cut., Striated cuticle. U.P., Unpaired papilla.

Fig. 1.—Anterior portion of the male specimen showing the buccal cavity.

Fig. 2.—Anterior portion of the female specimen showing the buccal cavity.

Fig. 3.—Posterior portion of the male specimen showing the caudal alæ and papillæ.

Fig. 4.—Posterior portion of the female specimen.

Fig. 5.—Portion of the female specimen showing the female genital apparatus.

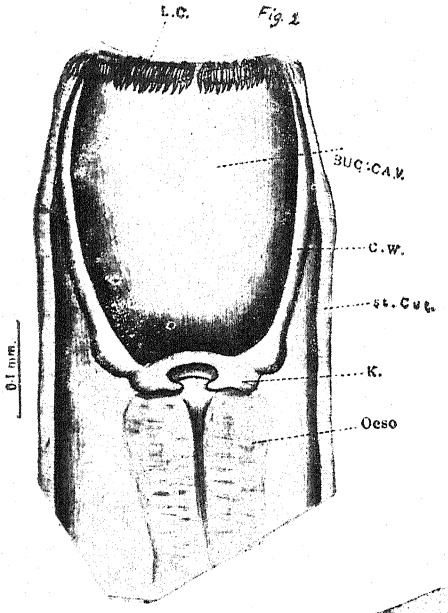

Fig. 1

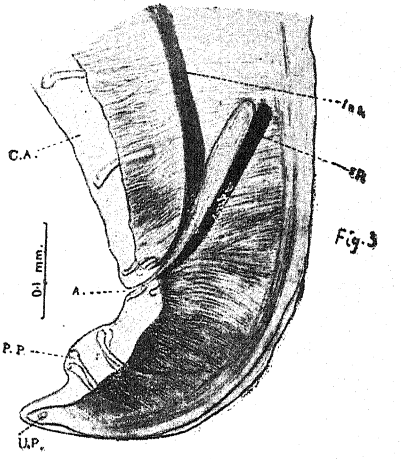

Fig. 3

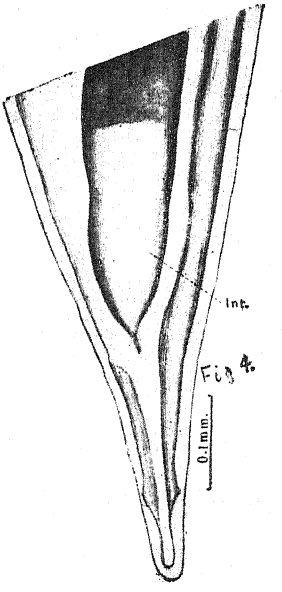

Fig. 4.

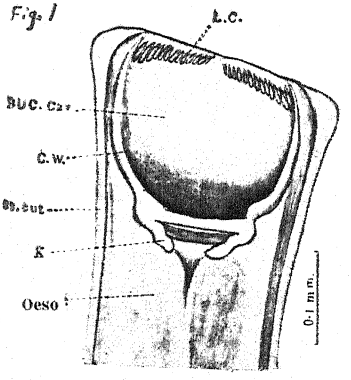

Fig. 1

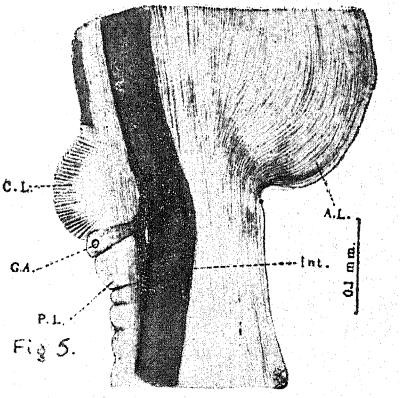

Fig 5.

CERCARIA ALLAHABADII N. SP.

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INTRODUCTION

The few species of snails available in the tanks and ponds near Allahabad, are not uncommonly infected with the larval trematodes, some of which are new to Science. The present paper embodies the account of one such species. The snails were carefully collected, identified and kept under proper conditions in the laboratory for the study of cercariæ which were taken out from time to time.

The present work was suggested by Dr. H. R. Mehra, M.Sc., Ph.D. (Cantab), and has been carried on under his close guidance. It is, therefore, my pleasant duty to express my indebtedness to Dr. H. R. Mehra for valuable help and advice. I also thank Dr. D. R. Bhattacharya, Ḥead of the Zoology Department for kindly providing me with the necessary facilities in the Department for the successful completion of this work.

OBSERVATIONS

Indoplanorbis exustus (Deshayes), the common Indian Snail harbours at Allahabad, during the winter months one of the Furcocerous cercariæ which resembles closely *Cercariae Indicae XXII* Sewell, but the differences are prominent enough to distinguish it as a new species. Of the snails examined during the months of January and February about 80 % were infected with this cercaria, whereas those opened in

December and the early part of March showed a gradual fall in infection. The pathological effect on the host was observed in the degenerated condition of the liver, which was closely packed with a huge number of sporocysts at every stage of development. The sporocysts attain a length of several millimetres; their free ends exhibit independent movement unlike those of the xiphido-cercariæ.

The cercaria is larger than *Cercariae Indicae XXII* Sewell. The length of the body when extended, the main stem and the ramus are equal, each measuring 0·2 mm. The width of the main stem is uniform throughout and measures 0·027 mm. The greatest width of the body is in the region anterior to the acetabulum and is 0·04 mm. The width of the body at the point of junction with the tail is equal to that of the main stem.

The cercaria swims very rapidly in water by lashing movements of its tail and body. Like most Furcocerous cercariæ it draws its body behind its tail by continuous flapping-movements of the furcal rami using them like the wings of a bird. This rapid movement is alternately followed with a short period of rest when at times the cercaria fixes itself on the bottom of a glass vessel by the oral sucker, so that the whole body is capable of making a slow sidewise movement.

The body is capable of considerable degree of extension and contraction and when fully extended it measures almost twice the size of that in the contracted condition. The circular muscle fibres lying beneath the cuticle do not cause that extreme contraction in the body which we notice in the tail, therefore the outline of the body does not take a crenated appearance even when it is fully extended as in *Cercariae Indicae XXII* Sewell. The oral apparatus acts both as a sucker and a penetrating organ. When it temporarily acts as a sucker, the protrusible snout crowned with a row of anteriorly directed solid spines is retracted

within the rest of the organ so as to assume a cuplike shape which helps it to fix itself to the substratum. The rounded anterior end of the body is also armed with several rows of solid spines extending as far back as the level of the oral apparatus. On the ventral aspect of the oral apparatus the four salivary ducts traverse its substance to open at the tip of the snout, while on its dorsal surface there are present two pyriform cells with finely granular protoplasm and small clear rounded nuclei in their distal end. Sewell considers these gland cells to be similar to the head-glands of the Schistosome cercariæ.

The body and the tail of the cercariæ are long and narrow. The greatest body-width lies in front of the acetabulum, in front of which the body tapers slightly towards the anterior end; while in the posterior region the body is almost of the same uniform breadth. The main stem of the tail has the same breadth throughout. It is annulated on account of extreme contraction of the circular muscle fibres lying beneath the cutis. The furcal rami are horn-shaped and are almost as long as the main stem. The ramus is not constricted off from the main stem at its point of origin, where it is almost half as wide as the latter. The union of the tail stem with the body is evidently a strong one, as decaudation takes place after a long time when the animal is subjected to increasing pressure under a coverslip. When confined under a coverslip, the cercaria progresses by the alternate use of its two suckers. The acetabulum is situated more towards the hinder region and occupies here about half the breadth of the body. The surface of the former is crowned with well-developed solid hook-like spines about 50 in number, which help the animal in gripping tightly to its secondary host.

There are two sets of muscle fibres in connection with the acetabulum. Circular fibres are especially prominent around the non-nucleated distal part, while powerful bands

also radiate from the proximal region to the adjacent parts of the dorsal side of the body where they are inserted.

The "*penetration gland cells*" are four in number. They lie anterior to the ventral sucker as in *Cercariae Indicae XXII* Sewell, but their arrangement is somewhat different from that of the latter. The first two cells instead of being situated at the same level lie one behind the other, whereas the second pair of cells occupies the same level. Each of these cells is provided with a clear nucleus in the centre, coarsely granular protoplasm around the periphery and a narrow duct passing forward to the ventro-lateral aspect of the penetrating organ. Of these four ducts the first and the third open on one side whereas the second and the fourth on the other.

Several views have been put forward about the nomenclature of these gland cells on account of the different functions assigned to them, such as salivary, poison, cephalic, digestive, mucus, mucin, salivary-mucin, periacetabular and secretory glands. According to Miller (1926), these gland cells are concerned with the dissolution of host tissues. He agrees with the conclusion recorded by the authors Faust and Meleney, and Szidut, that these glands disappear in the mammalian Schistosomes and in certain Holostomes soon after penetration of the skin of the definitive or intermediate host, and in consequence he prefers to call them the '*penetration gland cells.*'

The mouth is small and terminal; it leads into the cavity of the oral sucker formed by the retraction of the protrusible snout within the oral apparatus. When the snout is retracted into a sucker the solid spines present on it border the inner margin of the latter. Behind the oral apparatus the pharynx is seen to be present as a bulbular enlargement of the œsophagus. Unlike that of the other cercariæ and adult trematodes it is not provided with muscle fibres. It is more clearly seen in older specimens

than in young ones. The narrow œsophagus continues to the anterior region of the first salivary gland cell, where it bifurcates into two narrow cæca, which enclose the first two gland cells and then run backward through the narrow intervening spaces between the second and the third and the second and the fourth of these cells. They then pass around the acetabulum terminating in two enlarged saccular structures one on each side apparently divided into three chambers comparable to the "*refractile bodies*" of Sewell. Sewell's description of *Cercariae Indicae XXII* is not clear about the exact nature of these '*refractile bodies*' to study which more closely the cercariæ were vitally stained with 1/500 Neutral Red in 6/1000 salt solution. The cæca, œsophagus and gland cells were uniformly stained proving beyond doubt that the so-called "*refractile bodies*" are nothing but the terminal expanded portions of the cæca. Moreover their refractile appearance is due to the colour of the fluid contained within them. The food in this cercaria does not consist of granular substance as in *Cercaria palustris* which will be described later but it is of the nature of refractile fluid substance.

The excretory system is of peculiar interest. Cort's statement that the "fundamental homology of excretory system of the forked-tailed cercariæ indicate that at least in this group the pattern of the excretory system is very conservative" holds good in the case of the body, though in that of the tail in this species, there appears to be a remarkable difference from the observations recorded before in this group.

The excretory vesicle is tripartite. The anterior arms gradually become narrow to become the main lateral collecting tubes, which bend and lie close against the outer margin of the cæca, till at about the middle of the acetabulum each divides into an anterior and posterior collecting tubules. The anterior pair of collecting tubules passes

forward and receives capillaries from three pairs of flame-cells while the posterior ones drain three pairs of cells lying in the posterior region, of which the first and the second pair lie very close to each other.

It is in the presence of this second pair of flame-cells that the excretory system in the body of this cercaria differs from that of *Cercariae Indicae XXII* Sewell, otherwise in the position of the flame-cells and the arrangement of the collecting tubes there seems to be a close similarity.

The excretory system in the tail of this cercaria shows a great dissimilarity from that of *Cercariae Indicae XXII* Sewell. It may be that this dissimilarity is not real and probably due to lack of proper observations.

The main caudal tube arises from the posterior end of the bladder and passes along the axis of the tail-stem. On its way it gives off a series of transverse branches forming annular loops, usually five in number on each side which are joined together by secondary ducts or longitudinal ducts, one on each side, running close to the inner margin of the tail. I have failed to trace out any connections of the longitudinal ducts in the tail with the collecting tubules of the body except through the main caudal duct.

The system of net-work in the tail thus formed by the intertwining of the various excretory tubules is capable of extension and retraction owing to the changes in size of the body brought about by its expansion and contraction, while the size of the main stem, however, remains unaffected.

At its distal end the main caudal tube divides into two branches, which pass along the furcal rami for about one-third of their length and then open to the exterior on their outer margin.

The flame-cells in the tail are four in number and appear to be more or less in a regular line over the main caudal tube at unequal distances. The connections of these

flame-cells with the tubules is a point which I have not been able to clear so far. What appears to me is that they are connected by fine ducts with the annular loops at their point of union with the main duct. It appears to be certain that they are not directly connected by fine tubules with the excretory system of the body as has been shown to be the case by Sewell in *Cercariae Indicae XXII* Sewell. The '*refractile bodies*' in the tail comparable to similar bodies in *Cercariae Indicae XXII* Sewell are nothing but spaces between these transverse ducts forming loops.

The genital organs are, however, not visible. It appears that the germinal epithelium in the cercariæ or in the sporocysts is not differentiated and localised to form the genital cells in any region. Longitudinal sections of the cercariæ fixed in Bouin's fluid and stained in Iron alum hæmatoxylin when examined failed to give any indication of the presence of these cells.

Development occurs in sporocysts. The sporocysts are threadlike structures one end of which is embedded in the liver mass of the host, while the other end is free and exhibits independent movements.

The mature sporocysts are sometimes more than one centimetre in length and are covered over by orange-coloured patches of the liver tissue of the host. Their fixed end is rounded and the free end slightly tapering. More than two hundred cercariæ come out of one sporocyst through its tapering end. Those coming out of the same sporocyst are at various stages of development. The simplest stage is a rounded mass of cells enclosed within an outer covering, *i.e.*, the cyst wall. The spherical mass then gradually becomes elongated and assumes the oval shape, the anterior end of which is somewhat rounded while the other end is more conical. A small part of the cellular mass about one-fourth of its length is cut off from the main mass by the extensions of the wall of the capsule as shown in Fig. 5 forming the

rudiment of the tail. The tail-end of the posterior division becomes bifid by a median depression representing the rudiments of the rami. The main mass by further development and growth forms the body of the cercaria and the small bifid mass gives rise to the stem of the tail with its furcal rami.

DISCUSSION

Before discussing the systematic position of this cercaria it is first necessary to mention briefly the different views about the classification of the "*Furcocerous cercariae*" suggested from time to time. Though work on this line was begun as early as the end of the 18th century yet no special attempt was made to classify this group of cercariæ till recently. Cort (1917) suggested a scheme on the basis of his study of six Furcocerous cercariæ. He laid special emphasis on the excretory system as the important criterion in classification. He writes: "a more complete knowledge of this system will do much to clear up relationships and to establish natural families. Also an increased knowledge of the excretory system of little known types of cercariæ will be of great help in solving life-histories by suggesting the groups of adults to which such forms belong."

Sewell's classification (1922) is based on an extensive study of the Furcocerous cercariæ. He admits of a closer affinity of the "Monostome Furcocerous cercariæ" with the "Distome Furcocerous cercariæ" than the relation of the former with other Monostome cercariæ; nevertheless he separates the two groups. He modifies the classification of the "Distome Furcocerous cercariæ" as given by Cort in the point that he combines his groups I and II into one, whereas he divides his III group into two groups. Group I of Sewell includes the Brevifurcate Apharyngeal forms divided into two series mainly depending upon the presence or absence of hollow spines and the fin-folds on the furcal rami. Group II includes the Longifurcate cercariæ, divided

into two series depending on the presence or absence of pharynx. Group III is meant to contain some peculiar forms with leaf-like appearance.

Faust (1924), however, includes the Monostomes and Distomes Furcocerous cercariæ into one homogeneous group, and lays much stress (even more than Cort does) on the excretory system to establish the natural groupings. He writes: "A study of the larval characters of the trematodes for some years has brought me to the conclusion that there is only one common system carried over from the cercaria to the adult, which is sufficiently definite and conservative as to be utilizable for purposes of group identification. That system is the excretory system. The more work that is done in this system, the more indicative it is of possessing value as a natural basis of classification, and the more evident is the artificiality of some of Lühe's groupings of larval forms and of the equal artificiality of some of the families of adult trematodes that have been created."

The most recent system of classification of these cercariæ is by Miller (1926) who attaches great importance to the presence and absence of pharynx. He states: "The presence or absence of a pharynx is the first consideration for the division of the Furcocerous larvæ into two main groups." He supports his view by Stunkard's (1923) studies on adult blood-inhabiting trematodes from reptiles and their relationships with the forms found in fishes, reptiles, birds and mammals, and writes: "These studies indicate that the blood flukes constitute a natural group." They on the other hand, lack a muscular pharynx and so it seems that the presence or absence of pharynx is of great significance and the possession or lack of a ventral sucker is relatively unimportant.

Miller (1926) divides these two main groups—*Pharyngeal* and *Apharyngeal*—into two sub-groups *Brevifurcates* and *Longifurcates*. Each of these he further divides into the

Monostome and Distome groups, except the *Pharyngeal Brevifurcates* in which no monostome cercaria has yet been found.

The cercaria under discussion has a structure which is more a bulbular enlargement of the gut than a real muscular pharynx.

According to Miller's scheme of classification *Cercaria allahabadii* can only be assigned to the group of the *Apharyngeal Longifurcate* forms which it resembles in the following features:—

(1) Furcæ longer than half the length of the tail-stem and not constricted off from the latter.
(2) The diameter of the tail-stem equals that of the body when fully extended and the attachment of the former is terminal to the latter.
(3) Absence of fin-folds on the furcæ.
(4) Body provided with parenchyme cells.
(5) Eye-spots absent.
(6) Anterior organ is less highly modified as an oral sucker.
(7) Ventral sucker large, and has a greater diameter than the anterior organ.
(8) Number of penetration-glands small in proportion to body and no differentiation into anterior coarsely granular and posterior finely granular cells.
(9) Presence of solid piercing spines.
(10) Flame cells in the tail-stem four in number.
(11) Wall of the tail-stem annulated.
(12) Tail-stem devoid of spines.
(13) Well-developed cæca reaching almost to the posterior end of the body.

The characters mentioned above are only peculiar to Longifurcate larvæ and distinguish them from the Brevifur-

cate forms. The distinction between the *apharyngeal* and the *pharyngeal forms* is, however, not so sharp.

Though Miller attaches much importance to the presence and absence of pharynx, nevertheless he points out the lack of rigidity in his scheme. He in his treatise of the *Apharyngeal cercariae* states: "It is quite probable that some of the larvæ included under this heading will be found, upon more careful study, to possess pharynges." The present form shows some affinity with the *pharyngeal longifurcate* cercariæ in the fact that its dilatation of the œsophagus referred to above indicates the rudimentary nature of its pharynx.

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EXPLANATION OF FIGURES IN THE PLATE

Fig. 1. *Cercaria allahabadii*—Ventral aspect.
(Measurements given according to scale.)

Fig. 2. Sporocyst—Entire view.
(Semi-diagrammatic.)

Fig. 3. Longitudinal section of the body of the cercaria.
(Measurements given according to scale.)

Fig. 4. Transverse section of the cercaria in the region of intestinal cæca.
(Measurements given according to scale.)

Fig. 5. Stages in the life-history of the cercaria.
(Diagrammatic.)

EXPLANATION OF LETTERS

Acetabulum.
Anterior collecting tubule.
Anterior end.
Caudal canal.
Cercaria.
Excretory bladder.
Excretory opening.
Furca.
Flame cell.
Head-gland.
Intestinal cæcum.
Longitudinal canal.
Main excretory canal.
Opening of the caudal canal.
Oesophagus.
Penetrating organ.
Pharynx.
Penetration gland cell.
Posterior collecting tubule
Posterior end.
Refractile fluid.
Spines.
Salivary duct.
Transverse loop.
Tail-stem.

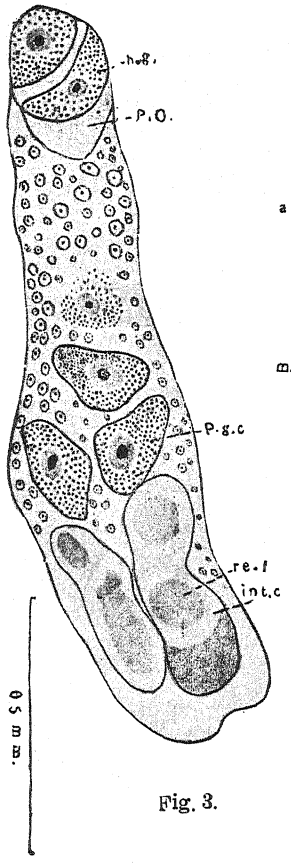

Fig. 3.

Fig. 1.

Fig. 2.

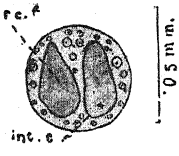

Fig. 4.

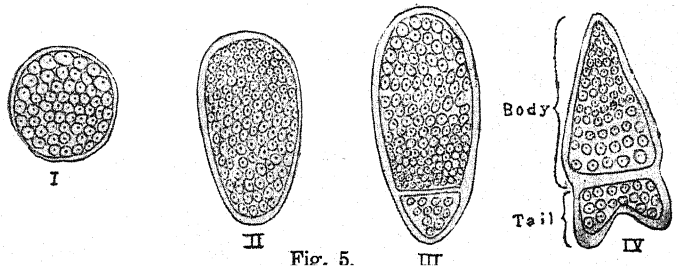

Fig. 5.

SECTION II

CHEMISTRY

INFLUENCE OF TEMPERATURE ON THE COAGULATION OF SOLS AND THE PROBLEM OF ACCLIMATISATION OF ANIMALS

BY

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In publications from these laboratories (J. Phys. Chem., 1926, *30*, 378; 480), we are trying to throw light on the problem of old age and acclimatisation. We have advanced the view that old age is associated with marked decrease in the catalytic activity of the body enzymes and cells. Consequently in old age there is an appreciable decrease in the metabolism of the animal body. Observations on the metabolism of human beings of different ages show that the metabolism expressed per square metre or kilogram is less in old age than in childhood or youth. Animal life is assumed to depend essentially on the activity of the cells or the enzymes. We have tried to prove that the phenomenon of ageing is common both to inorganic and organic colloids and precipitates. We have shown that the activity, adsorptive power, stability and viscosity of hydrophobe colloids in general, decrease and electric conductivity increases with time. On the other hand with hydrophile colloids the viscosity and the amount of hydration increases up to a limiting value while the electric conductivity decreases on ageing. We have recently shown that colloids can be divided into two classes according to their behaviour on exposure to light. Thus sols of ferric hydroxide, chromium hydroxide, zirconium hydroxide, ceric hydroxide, vanadium pentoxide, arsenious sulphide and manganese dioxide become less stable on exposure to light.

On the other hand sols of prussian blue, cupric ferrocyanide, mastic and gum dammar are stabilised when exposed to light.

In this communication we are recording the results obtained on the influence of temperature on the coagulation of sols.

The coagulation of several sols was investigated by placing the sol and the electrolyte in thermostats at constant temperature. The following results were obtained:—

FERRIC HYDROXIDE SOL

In order to prepare ferric hydroxide sol small quantities of ammonium carbonate were added to concentrated solution of ferric chloride, till the precipitate of ferric hydroxide redissolved. The sol was dialysed for six weeks.

TABLE I

Concentration of sol = 26·745 grms. of Fe_2O_3 per litre.
Amount of sol taken each time = 2 c.c.
Total volume = 10 c. c. ; Time = 1 hour.

Electrolyte.	Amount to coagulate in c. c.			
	at 20°C.		at 60°C.	
Potassium chloride N/5	1·1	15·7	0·90	15·65
Potassium sulphate N/100	1·4		1·15	

CUPRIC FERROCYANIDE SOL

Dilute solutions of cupric sulphate and potassium ferrocyanide were mixed, potassium ferrocyanide being in slight excess and the mixture was dialysed for 10 days, with occasional stirring.

TABLE II

Concentration of sol = 3·1 grms. per litre.
Amount of sol taken each time = 2 c.c. (A-sol);
1 c.c. (A/2-sol).

Total volume = 10 c.c.; Time = 1 hour.

Electrolyte.	Amount to coagulate in c.c.			
	A sol		A/2 sol	
	at 30° C.	at 60° C.	at 30°C.	at 60°C.
Potassium chloride N/4	3·2 } 94	2·0 } 80	3·5	2·4
Barium chloride N/200	1·7 }	1·25 }	1·9	1·2
Hydrochloric acid N/4	2·6	2·0	2·7	2·25

PRUSSIAN BLUE SOL

In order to prepare prussian blue sol, dilute solutions of ferric chloride and potassium ferrocyanide were carefully mixed and a small quantity of ammonium oxalate was added as a peptising agent. This mixture was subjected to dialysis for twelve days and a clear deep blue sol of prussian blue was obtained.

TABLE III.

Concentration of the sol = 9·33 grms. per litre.

Amount of sol taken each time = 1 c.c. (A-sol); 0·5 c.c. (A/2—sol).

Total volume = 10 c.c.; Time = 1 hour.

Electrolyte.	Amount to coagulate in c.c.			
	A sol.		A/2 sol.	
	at 30° C.	at 60° C.	at 30° C.	at 60° C.
Potassium chloride N/4	2·45 } 53	2·95 } 73·7	3·1	3·5
Barium chloride N/200	2·3 }	2·0 }	1·9	1·85
Hydrochloric acid N/4	...	...	2·1	0·4

VANADIUM PENTOXIDE

The sol was prepared by taking finely powdered ammonium vanadate in mortar, to which a concentrated solution of hydrochloric acid was slowly added till no more of red vanadic acid precipitated. The precipitate was allowed to settle and the clear liquid was decanted. The precipitate was washed three or four times by decantation with distilled water in order to free it from ammonium chloride. At this stage, the precipitate has a tendency to pass into the colloidal state. The precipitate was now vigorously shaken in a coloured glass bottle with distilled water and a clear deep red coloured sol of vanadium pentoxide was obtained.

TABLE IV

Concentration of the sol = 3·64 grms. V_2O_5 per litre.
Amount of sol taken each time = 1 c. c. (A-sol); 0·5 c. c. (A/2 sol).
Total volume = 10 c. c. ; Time = 1 hour.

Electrolyte	Amount to coagulate in c. c.			
	A sol		A/2 sol	
	at 30°C.	at 60°C.	at 30°C	at 60°C.
Potassium chloride N/10	0·95 } 35	0·75 } 39	0·8	0·65
Barium chloride N/500	1·35 }	0·95 }	1·15	0·70
Aluminium nitrate N/2000	1·3	0·95	0·8	0·55

STANNIC OXIDE SOL

Stannic hydroxide was precipitated by the action of an excess of ammonium hydroxide on stannic chloride solution. The precipitate on washing once or twice with

water passes into a negatively charged clear sol, which was purified by dialysis.

TABLE V

Concentration of the sol = 6·66 grms. of SnO_2 per litre.
Amount of sol taken each time = 1 c. c.
Total volume = 10 c. c. ; Time = 1 hour.

Electrolyte	Amount to coagulate in c. c	
	at 30°C.	at 60°C.
Potassium chloride N/4	2·4	1·4
Barium chloride N/200	1·1 } 109	0·85 } 82

ZIRCONIUM HYDROXIDE SOL

A solution of zirconium nitrate was boiled vigorously for half an hour and was dialysed for 10 days and a clear sol was obtained.

TABLE VI

Concentration of the sol = 9·56 grms. of ZrO_2 per litre.
Amount of sol taken each time = 2 c. c. (A-sol) ; 1 c. c. (A/2 sol).
Total volume = 10 c.c. ; Time = 1 hour.

Electrolyte	Amount to coagulate in c. c.			
	A sol.		A/2 sol.	
	at 30°C.	at 60°C.	at 30°C.	at 60°C.
Potassium chloride N/4	1·1	0·4	1·5	0·7
Potassium sulphate N/200	1·9 } 29	1·55 } 13	—	—

ZIRCONIUM HYDROXIDE SOL (IN COLD)

A clear aqueous solution of zirconium nitrate prepared at the ordinary temperature was dialysed for 10 days and a clear sol was obtained.

TABLE VII

Concentration of the sol = 9·42 grms. of ZrO_2 per litre.
Amount of sol taken each time = 2 c.c. (A-sol) ; 1 c. c. (A/2 sol).
Total volume 10 c.c. ; Time 1 hour.

Electrolyte	Amount to coagulate in c.c.			
	A sol		A/2 sol	
	at 30°C.	at 60°C.	at 30°C.	at 60°C.
Potassium chloride N/4	1·9	0·9	1·8	0·95
Sodium citrate 0·0032 M	2·0	1·6	1·3	1·2

ALUMINIUM HYDROXIDE SOL

A solution of aluminium nitrate containing an excess of sodium acetate was dialysed for 20 days. A clear dilute sol of aluminium hydroxide was obtained.

TABLE VIII

Concentration of the sol = 1·32 grms. of Al_2O_3 per litre.
Amount of sol taken each time = 4 c.c. (A-sol); 2 c.c. (A/2-sol)
Total volume = 10 c.c.; Time = 1 hour.

Electrolyte	Amount to coagulate in c.c.			
	A-sol		A/2 sol	
	at 30°C.	at 60°C.	at 30°C.	at 60°C.
Sodium citrate 0·0159 M	0·8	0·6	0·4	0·4
Sodium tartrate 0·019 M	0·9	0·9	0·5	0·5

CHROMIUM HYDROXIDE SOL

This sol was prepared by the action of ammonium carbonate on a solution of chromium-tri-chloride, till the precipitate of chromium hydroxide formed redissolved. The sol was allowed to dialyse for 17 days.

TABLE IX

Concentration of the sol = 1·725 grms. of Cr_2O_3 per litre.
Amount of sol taken each time = 2 c. c. (A-sol) ; 1 c. c. (A/2 sol).
Total volume = 10 c. c ; Time = 1 hour.

Electrolyte.	Amount to coagulate in c. c.			
	A sol.		A/2 sol.	
	at 30°C.	at 60°C.	at 30°C.	at 60°C.
Potassium sulphate N/200.	3·7	3·4	2·6	2·1
Sodium citrate 0·0032 M.	1·2	1·1	0·75	0·65

CERIC HYDROXIDE SOL

25 grms. of ceric-ammonium nitrate were dissolved in 250 c. c. of water at 25°C. The solution was dialysed for 6 days after filtering.

TABLE X

Concentration of the sol = 17·65 grms. of CeO_2 per litre.
Volume of the mixture = 10 c. c.

Amount of sol taken each time = 1 c. c.; Time = 1 hour.

Electrolyte at 60°C.	Amount to coagulate in c. c.			
	at 30°C.		at 60°C.	
Potassium chloride N/4.	4·8	171	2·1	75
Potassium sulphate N/200.	1·4		1·4	

ARSENIC SULPHIDE SOL

The sol was prepared by passing a slow current of H_2S in a solution of arsenious oxide, the excess of H_2S was removed by passing hydrogen.

TABLE XI

Concentration of sol = 26·73 grms. of As_2S_3 per litre.
Amount of sol taken each time = 2 c.c.
Total volume = 10 c.c.; Time = 1 hour.

Electrolyte.	Amount to coagulate in c. c.		
	at 30°C.	at 60°C.	at 80°C.
Potassium chloride N/4.	2·6	2·8	8·2
Barium chloride N/50.	1·2	1·1	0·9
Sulphuric acid 0·65 N.	1·05	1·05	1·35

SHEEP SERUM

Fresh sheep-blood was taken in a bottle which formed a firm clot within a few minutes. After a short time, syneresis took place and straw-coloured clear serum was squeezed out, which was used for experiments.

TABLE XII

Amount of serum taken each time = 2 c.c.
Volume = 10 c.c.; Time = 1 hour.

Electrolyte.	Amount to coagulate in c. c.	
	at 30°C.	at 60°C.
Hydrochloride acid N/100	3·55	4·35
Sulphuric acid N/100 ...	2·80	3·35
Oxalic acid N/100 ...	3·3	3·50
Acetic acid N/50 ...	1·9	2·3
Potassium fluoride 8 N ...	1·85	1·9
Sodium tartrate 1·91 M ...	7·15	7·1

DAMMARHARZ SOL

A concentrated solution of dammarharz was prepared in alcohol. The alcoholic solution was poured into distilled water, and the sol thus obtained was dialysed for seven days.

TABLE XIII

Concentration of the sol = 4·44 grms. per litre.
Amount of sol taken each time = 1 c.c.
Total volume = 5 c.c.; Time = 1 hour.

Electrolyte.	Amount to coagulate in c. c.		
	at 30°C.	at 60°C.	at 70°C.
Potassium chloride N/8 ...	1·1	1·3	1·5
Barium chloride N/8 ...	0·55	0·65	...
Hydrochloric acid N/100 ...	1·7	0·55	0·8

GAMBOGE SOL

A concentrated alcoholic solution of gamboge was poured into distilled water, and the sol thus obtained was dialysed for a week.

TABLE XIV

Concentration of the sol = 7·92 grms. per litre.
Amount of sol taken each time = 1 c.c.
Total volume = 10 c.c.; Time = 1 hour.

Electrolyte.	Amount to coagulate in c. c.		
	at 30°C.	at 40°C.	at 50°C.
Potassium chloride N ...	4·2	4·30	4·45
Barium chloride N/8 ...	1·0	1·20	1·30
Hydrochloric acid N/100	3·1	3·05	2·9
Tartaric acid N/10 ...	3·6	...	3·4
Oxalic acid N/10 ...	1·3	...	1·15

MASTIC SOL

A concentrated alcoholic solution of mastic was prepared and poured into distilled water. The sol thus obtained was dialysed for a week.

TABLE XV

Amount of sol taken each time = 2 c.c.
Total volume = 5 c.c.; Time = 1 hour.

Electrolyte.	Amount to coagulate in c. c.	
	at 30°C.	at 50°C.
Potassium chloride N ...	1·2	1·4
Barium chloride N/4 ...	0·75	0·9
Hydrochloric acid N/100 ...	1·2	0·95

The foregoing experimental results show that sols of ferric hydroxide, stannic hydroxide, zirconium hydroxide, (prepared in the hot and cold conditions), aluminium hydroxide, chromium hydroxide, cerium hydroxide and cupric ferrocyanide require smaller quantities of electrolytes when coagulated at higher temperatures than at 30°. In other words, the above sols become more aged and unstable when kept at higher temperatures. On the other hand, gamboge, gum dammar, mastic and sheep serum become stable when coagulated at higher temperatures.

We have proved that sols of ferric hydroxide, chromium hydroxide, aluminium hydroxide, stannic hydroxide, zirconium hydroxide, cupric ferrocyanide and cerium hydroxide become less stable towards electrolytes even when kept at the ordinary temperature, hence our experimental results show that as far as these sols are concerned, the influence of temperature leads to the accentuation of the time effect. These sols become unstable more readily when kept at a higher temperature than at the ordinary.

We have definitely proved that sols of mastic, gum dammar, gamboge, etc., are hydrolysed and the stability of these sols towards electrolytes increases with the degree of hydrolysis. At higher temperatures, these sols become more hydrolysed and their stability is also increased at higher temperatures. In presence of acids, the hydrolysis of these sols is greatly suppressed and the stability is decreased, hence in the coagulation of these sols by acids at higher temperatures, small quantities of acids are required.

In the case of prussian blue, the sol becomes more stable towards potassium chloride at higher temperatures, whilst it becomes less stable towards barium chloride and hydrochloric acid. It appears that cupric ferrocyanide becomes decomposed at higher temperatures and requires smaller quantities of electrolytes for coagulation.

In a previous communication (J. Phys. Chem. 1926, *30*, 480) we have applied Stefan's law of radiation in explaining the increase in metabolism observed when the surrounding temperature of a warm-blooded animal is lowered. We have advanced the view that the effect of transportation of a warm-blooded animal from a comparatively warmer climate to a very cold climate will be to activate the enzymes and cells in the body and it will lead to the shortening of the life of the animal. On the other hand, the transportation of a warm-blooded animal from a very cold climate to a comparatively warm country is to cause the body cells and enzymes work at a slower speed and the life of the animal may be prolonged by this transportation.

It is well known that cold-blooded animals live much longer than warm-blooded animals of the same size because the catalytic activity of the cells and the enzymes present in cold-blooded animals is not as great as those in warm-blooded animals. When a cold-blooded animal living in a warm country is taken to a cold-country, the metabolism will decrease and the animal leads a life of less intensity and greater duration. On the other hand, by the transportation of a cold-blooded animal from a cold to a warm country, the catalytic activity of the cells and enzymes is increased. This leads to a shortening of the life period of the animal.

From our experiments on the coagulation of sols it will be observed that the stability of several colloids decreases considerably by increasing the temperature of the colloid, and the colloids will age more rapidly. Consequently the colloids present in cold-blooded animals will have longer duration of healthy life, and it leads to the greater longevity of the animals themselves.

The body temperature of warm-blooded animals is normally much higher than the surrounding air. In the case of some birds, sparrow, hen, etc., the body temperature

is about 42°, in the case of rabbit 39·6° and in the case of a dog it is 39·2°. It will be clear from the results published in the foregoing pages that several body colloids, cells and enzymes present in these animals will age readily and become unstable at these moderately high temperatures. Moreover these cells and catalysts are made to work at a high speed in order to make up for the heat lost by radiation and other sources. Consequently the longevity of these warm-blooded animals cannot be as high as that of cold-blooded animals.

Voit gives the following results on the influence of temperature on the metabolism of a fasting man.

Temperature ...	4·4°	6·5°	9°	14·3°	16·2°	24·2°	26·7°	30°
CO_2 excreted in grams	201·7	206·0	192	155·1	158·3	166·5	160·0	170.6

It appears from the above results that when the outside temperature is about 20° the metabolism of the animal body is the minimum. Similar results showing that the metabolism is minimum when the external temperature is about 20°, have been obtained with other warm-blooded animals. We are of the opinion that the longevity of an animal is increased if the body cells and enzymes are made to work at a minimum speed. Moreover at the temperature 20° the body colloids do not age rapidly hence this temperature is the most suitable one for the healthy life of warm-blooded animals which maintain a higher body temperature than the surrounding air. People living in a country under suitable hygienic conditions should have the maximum longevity, if the average temperature is near about 20°.

SUMMARY

(1) Sols of ferric hydroxide, chromic hydroxide, vanadium pentoxide, aluminium hydroxide, stannic hydroxide, zirconium hydroxide and ceric hydroxide and cupric ferrocyanide require smaller quantities of electrolytes when coagulated at 60° than the amounts required for coagulation at 30°.

(2) The amount of salts required to coagulate sols of dammarharz, mastic, gamboge and prussian blue and sheep serum at 40°, 50°, 60° and 70° are greater than those required at 30°. When the coagulation is effected by hydrochloric acid, the amounts of acid required are smaller, the higher the temperature. These results can be explained from the viewpoint of the hydrolysis of the sols.

(3) Increase of temperature accentuates the ageing of these sols.

(4) An explanation has been advanced for the greater longevity of cold-blooded animals than warm-blooded animals of the same size.

(5) People living in a country having an average outside temperature 20° should have the maximum longevity, because when the outside temperature is about 20° the animal metabolism seems minimum.

ON AN EXPERIMENTAL STUDY IN HEMOLYSIS

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INTRODUCTION

A large number of workers have studied the hemolysis of red blood corpuscles by different substances[1] but as yet no clear explanation exists as to the mechanism of the change in the permeability of the corpuscle membrane under the influence of different hemolytes. That a single explanation may not cover all cases is evident from the fact that simply pure water will hemolyse the corpuscles which is entirely due to the absorption of water owing to a difference in the osmotic pressure inside and outside the cell membrane. The membrane is thus ruptured owing to a swelling of the whole cell.

According to modern conceptions the membrane of red blood corpuscles is a skeleton containing protein together with some lipoid materials. Hattori,[2] Gough[3] and others assumed that the membrane consists mainly of lecithin, and cholesterol. Pascucci[4] considers that it is a proteid permeable membrane with a large amount of lecithin, cholesterol and cerebroside. Since it is well-known that all the constituents

[1] Höber : Physikalische Chemie der zelle Ünd der gewebe, 1924, p. 499.

[2] Hattori : Biochem. Zeitschr., 1921, *119*, 45—64.

[3] Gough : Biochem. Zeitschr., 1924, *18*, 202—214.

[4] Pascucci : Beitr. Chem. Physiol. Path, *6*, 543—551, 552—566; cf. Bechhold's views on the composition of the membrane.

of the corpuscle membrane are colloidal in nature it is evident that the permeability of the membrane will be largely influenced by the action of substances which may affect the colloidal properties of any of the constituents. In the present paper a mechanism of hemolysis will be suggested on the basis of the colloidal properties of the membrane.

The present paper also contains an experimental study of hemolysis by various chemical hemolysers as well as by hemolytic serum. A study has also been made of the effect of mixtures of hemolytes and that of the action of normal serum in presence of other hemolysers. The experiments have been done both in saline as well as in sucrose solution.

EXPERIMENTAL METHOD

The method of experimentation depends upon the colorimetric determination of complete hemolysis. When the end-point was observed visually and when care was taken to select glass tube of the same bore and thickness and of equal transparency, practically concordant results could be obtained in duplicate experiments, the error being very slight. In all the experiments given in these pages, defibrinated sheep corpuscle washed three times with either normal saline or sucrose as the case may be to free it completely from serum, has been used. The solution of chemical hemolytes such as sodium taurocholate, potassium oleate and saponin were all prepared fresh, the substances being pure chemicals of either Merck or Kahlbaum. Since the experiments have been carried on through a long period it has been found necessary to use fresh samples of blood many times; consequently in different tables identical data on hemolysis will not be observed. The results of each table however are absolutely comparative and no difficulty will be experienced in the proper interpretation of the data. The usual method of carrying out an experiment has been to take a certain amount of the corpuscles in one test tube, and in another test tube a known

amount of hemolyte mixed with calculated quantity of normal saline or sucrose as the case may be to give a constant volume when the corpuscles and hemolyte solution were mixed together. The mixing was rapidly done three times, and the mixture was allowed to rest in an uniform temperature of 32° C and the time of complete hemolysis was then noted. The results obtained have been summarised and are given in different sections.

I. The Nature of the Time-Dilution Curves in Taurocholate Hemolysis.

In an early paper on the hemolytic action of bile derivatives, MacLean and Hutchinson[1] made some interesting observations on the hemolytic behaviour of the sodium salts of cholalic, choleic and glycocholic acids. They found that these substances are capable of producing hemolysis in the ordinary way when strong doses are used but exhibit marked peculiarities when present in considerably weaker amounts. It was found that under similar conditions the same hemolytic effect can be produced in a given time by widely divergent amount of the salt. Between these two points lies what may be termed a more or less neutral zone in which the hemolysis is very considerably delayed depending on the relative amount of the hemolytic agent employed. Ponder[2] in recent years has shown, using low concentration of taurocholate and cells that the time-dilution curve of taurocholate is of much simpler nature and does not show any abnormal behaviour like that of glycocholate. We have made a thorough study of sodium taurocholate as a hemolyte with different concentrations both of red blood corpuscles and of the hemolyte. Our results show that sodium taurocholate also shows a similar behaviour to that of glycocholate at higher concentrations; but at lower concentrations the time-dilution curve is perfectly

[1] MacLean and Hutchinson, Biochem. Journ., 4, 369, 1909.
[2] Ponder: Proc. Roy. Soc., B., *93*, 86, 1922.

normal. The results given in Table I have been obtained with 1 c. cm. of a 5% red blood corpuscles, the taurocholate concentration being 3% and the total volume 5 c. cm.

TABLE I

Taurocholate in c. cm.	Final Concentration	Time for complete hemolysis (in minutes)
0·1	1/1666	26
0·5	1/333	3
1·0	1/166	16
1·5	1/111	11
2·0	1/83·3	27½
2·5	1/66·6	42
3·0	1/55·6	27
3·5	1/47·6	5

In Table II are shown the results with lower concentrations of corpuscles as well as of hemolytes; the taurocholate concentration is 0·3 per cent and 1 c. cm. of an 1% corpuscles has been used.

TABLE II

Taurocholate in c.cm.	Final concentration	Time for complete hemolysis
0·1	1/16666	> two hours
0·2	1/8333	> two hours
0·3	1/5555	24 min.
0·4	1/4166	5 min.
0·5	1/3333	4 min.
0·6	1/2777	3 min. 15 sec.
0·7	1/2381	2 min. 20 sec.
0·8	1/2083	1 min. 30 sec.
0·9	1/1851	1 min. 7 sec.
1·0	1/1666	0 min. 55 sec.
1·2	1/1389	0 min. 40 sec.

In Fig. I, the results given in Tables I and II are plotted. Though the scales are different the nature of the curves will be immediately apparent from the graphical representation. It will be observed that with higher concentrations of red blood corpuscle and taurocholate the time-dilution curve shows a sort of periodicity while in low concentrations, the series is perfectly normal. We have discussed these results in a separate paper elsewhere but the interesting thing which should be noticed here is that taurocholate hemolysis is exactly similar in nature to that of glycocholate, choleate and cholalate hemolysis when comparatively higher concentrations of corpuscles and hemolyte are used. This also possibly explains some contradictory results to be found in the literature. Thus Ponder[1] found that glycocholate is a very weak hemolyte when compared to taurocholate but we have found previously[2] that glycocholate is a much stronger hemolyte than taurocholate. Thus it was observed by us that 1 c.c. of M/20 sodium glycocholate required one minute only for complete hemolysis of a certain quantity of corpuscles whereas under similar conditions one c.c. of M/20 sodium taurocholate required 42 minutes to effect this change. The difference between our results and that of Ponder probably depends upon the facts that we have been comparing the two hemolytes in concentrations far different from that used by Ponder. It is quite possible that in the concentrations we have been using, the comparison has been made in that portion of the curves where the action of taurocholate was about the minimum but the effect of glycocholate was near about the maximum. This would show that glycocholate is a stronger hemolytic agent. In the experiments of Ponder, however, the concentrations of the comparing hemolytes may have been such that the conditions are just

[1] Ponder : loc. cit.
[2] Sen and Basu : Journ. Ind. Chem. Soc., *5*, 1, 1928.

the reverse of that of ours; hence an entirely different result was obtained. It therefore appears that sodium glycocholate may have either a greater or lower hemolytic action than sodium taurocholate depending on the particular concentration range in which the comparison is made.

II. Effect of Mixture of Hemolytes

Though the study of hemolysis by single chemical hemolysers has been frequently made by different investigators, much investigation has not been carried out on hemolysis by mixtures of chemical hemolyte. In an early paper Moore, Wilson and Hutchinson[1] following the experiments of Sachs and Altmann[2] studied what they called the balancing action of certain pairs of hemolysers in preventing hemolysis and showed that there was an inhibiting action observed when sodium linoleate was used in conjunction with a pig serum both of which have a hemolytic action on sheep's corpuscles. They further observed that when a mixture of sodium oleate and linoleate was used no such inhibition was to be observed. A little earlier to this paper, Arrhenius[3] found that sodium oleate increases the action of cobra poison but diminishes that of saponin. We have made a detailed study of the hemolytic behaviour of mixtures of several chemical hemolytes and some results as well as the conclusions arrived at will be given here. In Table III, some results with a mixture of saponin and oleate are shown. The corpuscle-concentration has been one c.c. of an 1 per cent suspension and the total volume 5 c.c., the concentration of saponin being 1/1000 and that of oleate being 1/10000.

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[1] Moore, Wilson and Hutchinson: Biochem. Journ., *4*, 346, 1909.
[2] Sachs and Altmann: Berl-klin. Wochensch., pp. 494, 699, 1908.
[3] Arrhenius: Biochem. Zeitschr., *11*, 161, 1908.

TABLE III

Saponin in c.cm.	Time in minutes for hemolysis in presence of oleate c.cm.				
	0	0·1	0·2	0·3	0·4
0·0	...	No hemolysis in 1½ hours	No hemolysis in 1½ hours.	No hemolysis in 1½ hours.	80 min.
0·2	48	21	10 min. 35 sec.	3 min. 9 sec.	1 min. 10 sec.
0·3	19 min. 9 sec.	8 min. 5 sec.	2 min. 25 sec.	2 min. 0 sec.	0 min. 40 sec.
0·4	11 min. 10 sec.	4 min. 40 sec.	1 min. 34 sec.	1 min. 0 sec.	0 min. 35 sec.
0·5	6 min. 35 sec.	2 min. 4 sec.	1 min. 0 sec.	0 min. 46 sec.	...
0·6	4 min. 2 sec.	1 min. 32 sec.	0 min. 50 sec.	0 min. 27 sec.	...

The results given in the above table show that oleate has a definite effect on the hemolytic action of saponin. Thus it will be observed from all the results given above that the addition of oleate increases greatly the hemolytic action of saponin. This is contrary to the results reported to have been obtained by Arrhenius. Exactly similar results have been obtained with the mixtures of taurocholate and oleate, as well as of saponin and taurocholate. In every case, the action of mixture of hemolysers appears to be of an additive nature and in these particular cases there is no retarding action.

III. Effect of Acid and Alkali

While studying the hemolysis by mixtures of hemolytes we have also made a detailed study of the hemolytic action of some hemolysers in acid or alkaline solution and some interesting results have been obtained. It has been found that in certain cases acids accelerate whilst alkali retards the hemolytic action of certain hemolytes, whilst in some other

cases, a reverse effect is observed. Thus two series of results in presence of hydrochloric acid and caustic soda may be given here. The concentrations of corpuscles and hemolytes are the same as in the results given in Table III.

TABLE IV

Saponin in c.cm.	Time of hemolysis in presence of Hcl, Final concentration		
	0	N/10000	N/5000
0·0	...	No hemolysis in two hours.	No hemolysis in two hours.
0·2	21 min. 16 sec.	18 min. 22 sec.	4 min. 20 sec
0·3	10 min. 38 sec.	5 min. 53 sec.	1 min. 26 sec.
0·4	6 min. 27 sec.	2 min. 43 sec.	0 min. 49 sec.
0·5	2 min. 57 sec.	1 min. 17 sec.	0 min. 30 sec.
0·6	2 min. 6 sec.	1 min. 0 sec.	0 min. 20 sec.

TABLE V

Oleate in c.cm.	Time of hemolysis in presence of Hcl, Final concentration.	
	0	N/5000
0·0	...	No hemolysis in 2 hours.
0·4	34 min. 4 sec.	No hemolysis in 1 hour.
0·5	13 min. 50 sec.	15 min. 23 sec.
0·6	8 min. 1 sec.	12 min. 21 sec.
0·8	2 min. 45 sec.	10 min. 31 sec.
1·0	1 min. 40 sec.	9 min. 42 sec.

In Tables VI and VII the hemolytic action of the same hemolytes in alkaline solutions are shown. In these

results the concentrations of corpuscles were one c.c. of a 5% and 1% suspensions respectively, the total volume 5 c. c. saponin concentrations 1 in 1000 and oleate 1 in 10000.

TABLE VI

Concentration of Corpuscles = 5%

Saponin pure in c. cm.	Time of hemolysis in presence of NaOH, Final concentration	
	0	N/500
0·0	...	No hemolysis in two hours
0·5	50 min.	156
0·8	12 ,,	73
1·0	10 ,,	56
1·2	4 ,,	50
1·5	3 ,,	21

TABLE VII

Concentration of Corpuscles = 1%

Amount of oleate 1 in 10000, in c.c.	Time of hemolysis in presence of NaOH, Final concentration	
	0	N/2500 (0·2 c.c. of N/25 NaOH)
0·0	...	142 min. 0 sec.
0·2	No hemolysis in 4 hours.	80 min. 0 sec.
0·3	No hemolysis in 4 hours.	90 min. 0 sec.
0·4	95% hemolysis in 4 hours.	87 min. 51 sec.
0·5	130 min. 0 sec.	88 min. 43 sec.
0·8	18 min. 24 sec.	71 min. 10 sec.
1·0	15 min. 0 sec.	45 min. 0 sec.
1·2	13 min. 2 sec.	0 min. 34 sec.
1·5	11 min. 24 sec.	0 min. 36 sec.
2·0	8 min. 9 sec.	Immediate hemolysis.

The experimental results given in the foregoing Tables IV, V, VI, and VII, show that acids and alkali have a definite effect on hemolysis by saponin and oleate. Several years ago, Ponder[1] studied some cases of acceleration or

[1] Ponder : Proc. Roy. Soc., B., *99*, 461, 1926.

retardation of hemolysis and showed that in saponin hemolysis, certain acid substances like acetic acid, glutaminic acid and aspartic acid accelerate the hemolysis to a great extent while sodium hydroxide inhibits the hemolysis. Aspartic acid also accelerates hemolysis by bile salts and by soaps and is itself an hemolyser at higher concentrations. Since alkali retards hemolysis while acids accelerate, the obvious conclusion would be that the hemolysis is influenced by the hydrogen-ion concentrations of the solutions. That this is undoubtedly an important factor would be evident from the fact that corpuscles are hemolysed when P_h of the solution is below 5. With alkali there is a wide zone of resistance. As a matter of fact it was shown in a previous paper[1] that we can expect a retarding action on hemolysis by hydroxyl ions up to certain concentrations on theoretical grounds. The researches of Eggerth and of Mond are in this line. Eggerth[2] found that when washed sheep's corpuscle is suspended in solutions of P_h 5·2 or more acid, it becomes progressively more electropositive and this change is coincident with hemolysis. In investigating the hemolytic action of H and OH ions, Mond[3] found that a slight decrease in P_h from neutrality causes hemolysis but on the alkaline side of the neutral point there is a wide zone of resistance. This can be easily exemplified in the case of saponin where we have shown that traces of acid accelerate the hemolysis, while traces of alkali retard the hemolysis, though at higher concentrations, the alkali would itself hemolyse the corpuscles. With taurocholate an almost similar thing has also been observed. In the case of oleate however the case is very different. Acid inhibits the hemolysis by oleate and alkali has the opposite effect in certain concentrations of the oleate. The action of acid is undoubtedly due to

[1] Sen and Basu : Journ. Ind. Chem. Soc., *5*, 17, 1928.
[2] Eggerth : Journ. Gen. Physio. *6*, 587, 1924.
[3] Mond : Pflüg. Archir,, *208*, 57, 1925.

a chemical interaction with the oleate liberating oleic acid which is a less powerful hemolytic agent than the salt. For this reason there is a great retardation of hemolysis when a mixture of oleate and hydrochloric acid is used. Since this chemical action must also take place when taurocholate is mixed with acid it must be assumed that taurocholic acid is also a good hemolytic agent and its efficiency is comparable to that of the salt at least up to certain concentrations. So far as oleate is concerned, it is certain that the salt is the active hemolyser. Hence the peculiar effect of alkali in accelerating the hemolytic power of oleate in low concentrations may be due to a checking of the hydrolysis of the oleate into free acid which would otherwise take place in so dilute solutions in the absence of alkali. The accelerating effect of alkali with higher amounts of oleate cannot however be easily explained. In any case this peculiar behaviour of alkali seems not to have been observed by any one before and deserves further study. The effect of acids thus differs in marked way from that of the alkali when these actions of different hemolytes are considered. Thus in the case of saponin, a slight trace of acid markedly accelerates the hemolytic action while a trace of alkali in some cases considerably retards it. A similar thing also happens in the case of taurocholate.[1]

[1] In connection with this effect of alkali, an interesting thing may be mentioned here. It will be shown later on, that if normal serum is added to the corpuscles before the addition of taurocholate or oleate, an inhibition of hemolysis is observed. If however, the serum is added to a mixture of corpuscle and taurocholate or oleate, an accceleration of hemolysis can easily be obtained. An exactly similar thing has been noticed with alkali. Thus it has been shown that the presence of alkali before the addition of taurocholate, or oleate retards the hemolysis in some cases, but if the alkali is added to a mixture of the corpuscles and taurocholate or oleate under identical conditions, a great acceleration of hemolysis can be easily observed. This lends support to the view, that the accelerating effect of serums on taurocholate and oleate hemolysis to be discussed in subsequent pages, is due, at least in part, to the alkali-content of the serum.

IV. Effect of Normal Serum on the Hemolytic Behaviour of Chemical Hemolytes

It is known from a long time that normal serum inhibits the hemolytic action of saponin and bile salts; and different investigators such as Ransom,[1] or Bayer,[2] Eisler,[3] Liebermann,[4] Sellards,[5] Ponder,[6] etc., have investigated this inhibitory action of serum from different points of view. A very interesting phenomenon has been observed, it seems first by Sachs[7] that normal serum when added to a mixture of oleate and red blood cells accelerates instead of inhibiting the hemolysis. Ponder[8] has shown in recent years that in the case of taurocholate hemolysis there is an accelerating effect of normal serum when it is added to a mixture of taurocholate and red blood cells. But if the serum is added to the cells before the addition of taurocholate a great inhibition of hemolysis is observed. In a study of this acceleration or retardation of hemolysis by normal serum, it was shown by us[9] that Ponder's conclusions could be possible only under special conditions; and experiments carried by us with comparatively higher concentrations of red blood cells all showed that normal serum in every case inhibited the hemolysis whether it was added to the red cells before or after the addition of the taurocholate. Ponder has however suggested (private communication), that this acceleration is to be observed only under some particular concentration ranges of the reacting substances. Since no thorough investigation on this particular problem has been published as yet, we have made a

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[1] Ransom : Deutsch. Med. Wöch., pp. 194, 1901.
[2] Bayer : Biochem. Zeitschr., p. 368, 1907.
[3] Eisler : Zeit. f. Exper. Path., U. Ther., *3*, 296, 1906.
[4] Liebermann : Biochem. Zeitschr., *4*, 25, 1907.
[5] Sellards : Bull. of John Hopkins Hospital, *19*, 268, 1908.
[6] Ponder : Proc. Roy. Soc., B, *95*, 42, 1923.
[7] F. Sachs : Biochem. Zeitschr., *12*, 278, 1908.
[8] Ponder : Proc. Roy. Soc., B, *95*, 403, 1923.
[9] Sen and Sen : Journ. Ind. Chem. Soc., *5*, 261, 1928.

detailed study of this retardation and acceleration of hemolysis by normal serum and we have been able to confirm Ponder's suggestions in this line. Since this acceleration and retardation of hemolysis by normal serum must be of great theoretical importance we shall give below some of our results in details; in the following experiments, the concentration of cells is one per cent, taurocholate 0·3%, total volume 5 c.c. and concentration of serum is 1/10; the quantity of corpuscles has been varied in different experiments. All the experiments have been done in normal saline.

TABLE VIII

2 c.c. corpuscles are used in these experiments.

	Time for complete hemolysis.
(1) 2 c.c. taurocholate + 1 c.c. saline added half a minute after the addition of taurocholate ...	3 min. 2 sec.
(2) 2 c.c. taurocholate + 1 c.c. serum added half a minute after the addition of taurocholate ...	50 % hemolysis in 1 hour.
(3) 1·5 c.c. taurocholate + 1·5 saline added half a minute after the addition of taurocholate ...	17 min. 8 sec.
(4) 1·5 c.c. taurocholate + 1·5 c.c. serum added half a minute after the addition of taurocholate ...	No hemolysis in 1 hour.

TABLE IX

	Time for complete hemolysis.
(1) 1 c.c. cell + 0·5 c.c. taurocholate...	7 min. 20 sec.
(2) 1 c.c. cell + 0·1 c.c. serum added before + 0·5 c.c. taurocholate ...	122 min.
(3) 1 c.c. cell + 0·2 c.c. serum added before + 0·5 c.c. taurocholate ...	60 % hemolysis in 3½ hours.

	Time for complete hemolysis.
(4) 1 c.c. cell + 0·5 c.c. serum added before + 0·5 c.c. taurocholate ...	No hemolysis in 4 hours.
(5) 1 c.c. cell + 0·5 c.c. taurocholate + 0·1 c.c. serum added half a minute after the addition of taurocholate	57 min. 46 sec.
(6) 1 c.c. cell + 0·5 c.c. taurocholate + 0·2 c.c. serum added half a minute after the addition of taurocholate	63 min. 0 sec.
(7) 1 c.c. cell + 0·5 taurocholate + 0·5. c.c. serum added half a minute after the addition of taurocholate	1 min. 42 sec.

TABLE X

	Time for complete hemolysis.
(1) 0·5 c.c. cell + 0·5 c.c. taurocholate	3 min. 21 sec.
(2) 0·5 c.c. cell + 0·1. c.c. serum added before + 0·5 c.c. taurocholate	16 min. 16 sec.
(3) 0·5 c.c. cell + 0·2 c.c. serum added before + 0·5 c.c. taurocholate ...	No hemolysis in half an hour.
(4) 0·5 c.c. cell + 0·5 c.c. taurocholate + 0·1 c.c. serum added half a minute after the addition of taurocholate	14 min. 46 sec.
(5) 0·5 c.c. cell + 0·5 c.c. taurocholate + 0·2 c.c. serum added half a minute after the addition of taurocholate	22 min. 28 sec.
(6) 0·5 c.c. cell + 0·5 c.c. taurocholate + 0·5 c.c. serum added half a minute after the addition of taurocholate	1 min. 4 sec.

	Time for complete hemolysis.
(7) 0·5 c.c. cell + 0·5 c.c. taurocholate + 1 c.c. serum added half a minute after the addition of taurocholate	0 min. 32 sec.

In the following Tables the effect of serum on oleate hemolysis is shown; concentration of oleate is 1 in 10000, the other conditions being the same.

TABLE XI

	Time for complete hemolysis.
(1) 1 c.c. cell + 1 c.c. oleate + 1 c.c. saline added half a minute after the addition of oleate ...	2 min. 52 sec.
(2) 1 c.c. cell + 1 c.c. oleate + 0·1 c.c. serum added before the addition of oleate	10 min. 56 sec.
(3) 1 c.c. cell + 1 c.c. oleate + 0·1 c.c. serum added half a minute after the addition of oleate ...	10 min. 48 sec.
(4) 1 c.c. cell + 1 c.c. oleate + 0·5 c.c. serum added before	No hemolysis in 2 hours.
(5) 1 c.c. cell + 1 c.c. oleate + 0·5 c.c. serum added half a minute after the addition of the oleate ...	70 % hemolysis in 2 hours.
(6) 1 c.c. cell + 1 c.c. oleate + 1 c.c. serum added half a minute after the addition of oleate ...	90 % hemolysis in 2 hours.

TABLE XII

Amount of cells used each time = 0·2 c.c.

Amount of oleate used each time = 0·3 c.c.

Time of complete hemolysis when one c.c. saline was added to a mixture of corpuscles and oleate one minute after the addition of the oleate ... 6 min. 9 sec.

Numbers.	Amount of serum, added one minute after the addition of oleate, in c.cm.	Time of complete hemolysis.
(1)	0·1	146 min.
(2)	0·2	210 min.
(3)	0·3	180 min.
(4)	0·4	6 min. 42 sec.
(5)	0·5	5 min. 54 sec.
(6)	0·6	5 min. 1 sec.
(7)	0·7	4 min. 1 sec.
(8)	0·8	3 min. 28 sec.
(9)	0·9	3 min. 19 sec.
(10)	1·0	3 min. 10 sec.

In the following Tables XIII and XIV, some results are given showing the effeet of the time interval between addition of taurocholate to the cells and the addition of the serum in causing the observed acceleration of hemolysis; the experiments have been done in isotonic sucrose solution, which incidentally shows that sucrose solutions behave in an entirely similar manner to that of the saline solution. The concentration of corpuscles is one per cent, of which 0·2 c.c. has been used each time. The taurocholate concentration is 0·1 per cent, of which one c.c. has been used each time. Other conditions remain the same.

TABLE XIII

Amount of serum, added half a minute after the addition of taurocholate, in c.cm.	Time of complete hemolysis.
0·0	2 min. 20 sec.
0·1	12 min. 24 sec.
0·2	30 % hemolysis in one hour.
0·3	5 % hemolysis in one hour.
1·0	12 min. 56 sec.

TABLE XIV

Amount of serum, added one minute after the addition of taurocholate, in c.cm.	Time of complete hemolysis.
0·0	2 min. 20 sec.
0·1	7 min. 4 sec.
0·2	2 min. 54 sec.
0·3	2 min. 10 sec.
0·4	1 min. 26 sec.
0·5	1 min. 13 sec.
1·0	Immediate hemolysis

The data presented in the above Tables are very interesting. It will be observed that the addition of normal

serum to the red blood cells before the hemolyte is actually added always inhibits the hemolysis ; whereas when serum is added after the addition of the hemolyte we may get either an inhibition or an acceleration of hemolysis, depending on the particular concentration of the red blood cells, the hemolytes, the amount of serum added and in certain cases on the time interval after which the serum is added to the mixture of corpuscles and hemolyte. Thus it will be observed from Tables VIII, IX and X that with two c.c. corpuscles no acceleration of hemolysis could be observed when the serum was added half a minute after the addition of the taurocholate. When 1 c.c. corpuscle was used then also no acceleration of hemolysis could be observed by the addition of serum but when its concentration was less than 0·5 c.c. From Table IX, with 0·5 c.c. of cells it will be found that we have reached a concentration range in which both inhibition and acceleration of hemolysis could be obtained depending upon the quantity of serum added. A similar fact is to be noticed in the case of oleate from Tables XI and XII. The results in Tables X and XII are also very important inasmuch as they allow us to draw a complete and continuous curve of inhibition and acceleration of taurocholate and oleate hemolysis by the gradual addition of increasing amounts of normal serum to the mixture of corpuscle and hemolyte after a definite time interval. Two typical curves are given in Figs. II and III ; and an analysis of these curves will be attempted in a later paper. It will also be observed from Table XIII that even under similar conditions there was no acceleration of hemolysis when the normal serum was added half a minute after the addition of taurocholate but when the time interval was one minute as in Table XIV, an acceleration of hemolysis was observed under identical conditions of concentrations. These facts lead us to the conclusion that the effect of normal serum in hemolysis is in reality very complex.

V. Effect of Normally Hemolytic Serum in Presence of Chemical Hemolytes

While discussing the hemolytic action of mixtures of hemolytes, it was stated that an inhibiting action is observed when sodium linoleate is used in conjunction with a pig serum both of which have a hemolytic action on sheep's corpuscles. This experiment is of similar nature to that done by Sachs and Altmann (loc. cit.) who found that when sodium oleate was added in proper quantity to a strongly active hemolytic serum, no hemolysis resulted which was supposed to be due to the sodium oleate acting as an anti-complement. We have now made a detailed study of the effect of hemolytic serum both in active as well as in inactivated condition in presence of other chemical hemolysers such as saponin, taurocholate, oleate, acids and alkali. For these experiments human serum containing normarly hemolysins for sheep corpuscles has been used. In the following Tables the corpuscle concentration is 1 c.c. of a 0·2% suspension in saline, saponin concentration 1 in 10000, oleate 1 in 50000 and taurocholate 0·1 per cent, the total volume being 4 c.c.

TABLE XV

Saponin in c. cm.	Time in minutes of hemolysis in presence of hemolytic serum, c. cm.		
	0·0	0·1	1·0
0·0	...	...	$8\frac{1}{2}$
0·8	$13\frac{1}{2}$	No hemolysis in 2 hours	...
0·9	$11\frac{1}{4}$	Partial hemolysis in hours	...
1·0	9	70	$10\frac{3}{4}$
1·1	$6\frac{1}{4}$	16	$10\frac{3}{4}$
1·2	3	12	$10\frac{1}{2}$

TABLE XVI

Taurocholate in c.cm.	Time of complete hemolysis in presence of hemolytic serum, c. cm.		
	0·0	0·1	1·0
0·0	...	...	$1\frac{1}{2}$
0·8	$2\frac{1}{2}$	30 °/₀ hemolysis in $1\frac{1}{2}$ hours	...
1·0	$1\frac{1}{4}$	80 °/₀ hemolysis in $1\frac{1}{2}$ hours	$13\frac{1}{2}$

TABLE XVII

Amount of $\frac{N}{500}$Hcl in c. cm.	Time of complete hemolysis in presence of hemolytic serum in c. cm.		
	0·0	0·1	1·0
0·0	...	...	14 min.
0·8	4 min.	$8\frac{3}{4}$ min.	...
1·0	3 min.	3 min.	70 °/₀ hemolysis in 1 hour.
1·2	2 min.	2 min.	40 °/₀ hemolysis in 1 hour.

TABLE XVIII

Amount of $\frac{N}{25}$NaOH in c. cm.	Time for hemolysis in presence of hemolytic serum in c. cm.		
	0·0	0·1	1·0
0·0	...	...	14 min.
0·8	$4\frac{1}{4}$ min.	8 min.	...
1·0	$3\frac{1}{2}$ min.	$6\frac{1}{4}$ min.	$11\frac{1}{2}$ min.
1·2	$2\frac{3}{4}$ min.	5 min.	...

So long results with hemolytic serum have been given. In the following Table XIX, the effects of hemolytic serum and the inactivated serum prepared by heating one sample of the hemolytic serum for half an hour at 56°C, are compared in presence of taurocholate as hemolyte.

TABLE XIX

Amount of Taurocholate in c. cm.	Blank	Time for hemolysis in presence of 0·1 c. c. serum.	
		Hemolytic serum.	Complement-free hemolytic serum.
1·5	7 min.	Slight hemolysis in 30 min.	Slight hemolysis in 30 min.
2·0	3¼ min.	10¼ min.	13 min.
2·5	1¾ min.	5 min.	6¾ min.

The complement-free serum was tested and was found to have no hemolytic power when used alone.

From the results obtained with hemolytic serum in presence of other chemical hemolysers, it will be evident that the results of Sachs and Altmann with soaps can be extended to the cases of saponin, taurocholate, acids and alkali. It thus appears that though an hemolytic serum is capable of hemolysing red blood corpuscles when present alone, it behaves as an inhibiting agent when added in presence of other chemical hemolysers. Its qualitative behaviour is almost similar to that of normal serum. But quantitatively its action differs from that of normal serum inasmuch as its inhibiting action is very limited at higher concentrations whereas normal serum at higher concentrations has an almost complete inhibiting action. In comparing the effects of hemolytic serum and complement-free hemolytic serum in Table XIX, it will be interesting to note that inactivated hemolytic serum has a greater retarding action than the other.

VI. Hemolysis in Isotonic Sucrose

The results so far given and discussed have been done mainly in physiologically normal saline solution. In a recent Paper[1] it was shown by us that sucrose has an inhibiting action on taurocholate hemolysis as compared to experiments made in saline solution. Several years ago Ponder and Kennedy[2] observed an inhibition of Saponin Hemolysis in presence of sugar. Ponder and Yeager[3] have shown the same thing with regard to taurocholate hemolysis. It has been shown by us that sucrose has an inhibiting effect on oleate hemolysis also. We give below some data about the effect of sucrose on oleate hemolysis. The concentration of corpuscles is one c.c. of an one per cent suspension.

TABLE XX

Amount of oleate in c. cm.	Final concentration.	Time of complete hemolysis.	
		In isotonic saline.	In isotonic sucrose.
0·5	1/100000	13 min. 50 sec.	19 min. 31 sec.
0·6	1/83333	8 min. 1 sec.	15 min. 54 sec.
0·8	1/62500	2 min. 45 sec.	13 min. 5 sec.
1·0	1/50000	1 min. 40 sec.	11 min. 2 sec.

It will be thus observed that sugars have a definite retarding action on saponin, taurocholate and oleate hemolysis. Ponder and Yeager in their recent Paper (loc. cit.) have shown that the effect of sugars is mainly on the corpuscles themselves, a view already advanced by us previously. It was therefore desirable to find out whether hemolysis

[1] Sen and Sen: loc. cit.
[2] Ponder and Kennedy: Biochem. Journ., *20*, 237, 1926.
[3] Ponder and Yeager: Biochem. Journ., *22*, 703—710, 1928.

in sucrose solutions shows an analogous behaviour to that in saline solutions, so far as the different aspects of hemolysis already studied in saline solutions are concerned. To make a full investigation therefore we have made experiments on the nature of the time-dilution curves of taurocholate, saponin and oleate hemolysis with different concentration of blood and the hemolytic effect of normal serum on the acceleration and retardation of hemolysis, etc., in isotonic sucrose solutions. We have already given some data on the effect of normal serum in Tables XIII and XIV which show an identical behaviour to that obtained in saline solutions and almost analogous behaviour has also been observed in the case of taurocholate, saponin and oleate hemolysis and we have come to the conclusion that hemolysis in sucrose solution shows in all important respects a similar behaviour to that carried in saline solution, the only difference being a depressing action of sucrose on the hemolytic efficiency of different hemolyte by changing the nature of the corpuscle membrane to a certain extent.

VII. The Mechanism of Hemolysis

In the preliminary introduction it was observed that the membranes of the red blood corpuscles are composed of substances which are colloidal in nature and part of them such as the lipoids (lecithin, cholesterol, etc.), can be easily dispersed as hydrophylic and hydrophobic colloids in water. It has already been known from some time past that a solution of bile salts has a dissolving effect on lecithin and cholesterol. In a previous Paper[1] we have shown that soaps, bile salts and saponin peptise the lipoids easily; consequently we can assume that the effect of these substances which also lower the surface tension of water greatly, in hemolysis is due to a peptisation of the lipoidal constituent of the corpuscle membrane. It is of course true that the presence

[1] Sen and Basu : loc. cit., p. 10.

of the protein will have an effect on this peptisation. It is well-known that corpuscle membranes are slightly negatively charged in saline or sucrose, and Gough[1] has shown that the agglutinating power of different metallic ions in the case of sheep's corpuscles is in the order Ce 7 Th 7 Ca 7 K_4 Fe $(CN)_6$. It will be interesting at this place to draw an analogy with an inorganic colloid. Thus we have found that the coagulating power of these Cat ions on a copperferrocyanide solution is Ce 7 Th 7 Ca 7 K_4 Fe $(CN)_6$ beginning with the highest. Copperferrocyanide further offers a closer analogy in that it forms membranes with properties very similar to those of the corpuscle membrane.

Thus it is impermeable to sugars and to many substances which are quite analogous to the behaviour shown by the stroma. A recent investigation by Gourchot[2] has shown that the variable permeability of the copperferrocyanide membrane in presence of different substances is caused by the coagulation or the peptisation of the membrane and he has drawn attention to the fact that the change in permeability of many plant cells in presence of alcohols investigated by Czapek[3] and the results of Walden[4] with different organic acids are nothing but due to a coagulation of the membrane. If now a similar view is advanced to explain the permeability of the stroma, the following becomes obvious. We can consider that ordinarily the corpuscle membrane is in a peptised condition and consists of fine granular particles, the interspaces of which are filled in by the adsorbed aqueous medius and assumption exactly analogous to that of the copperferrocyanide membrane. Hence water and many water-soluble substances can pass through the membranes. Aqueous solution of some neutral salts is however not easily permeable

[1] Gough: loc. cit.
[2] Gourchot: Journ. Phys. Chem., *30*, 83, 1926.
[3] Czapek: Ber. Deutsch. Bot. Ges., *28*, 159, 1910.
[4] Walden: Zeit. physikal. Chem., *10*, 699, 1893.

because the stroma is polarised owing to the existence of an electrical charge. Salts which have a high coagulating power on the stroma may make the membrane permeable by coagulating it and forming coarse flakes but at the same time the hemoglobin may also be precipitated. That with a low concentration of strongly coagulating ions, a permanent change occurs in the stroma is shown by the results of Mikwa[1] who found that small amounts of uranyl acetate actually damage the structure of the cells as is shown by the increased sensitiveness to physiological saline. The impermeability of the sugars may however be due to a different cause, namely, due to negative adsorption, a fact well-known in the case of copperferrocyanide membrane.

We can therefore summarise the phenomenon of hemolysis as depending on the following several factors:—

(1) Coagulation of the corpuscle membrane whereby the membrane material will form coarse flakes and hence gives an increase in permeability.

(2) Peptisation of some of the membrane constituents such as the lipoids whereby the whole membrane will get loosened and an increase in permeability will occur.

(3) Mechanical or other forms of rupture such as due to swelling or imbibing of water either by the cell as a whole or by any component of the membrane.

(4) Pure solubility effect as for example in the dissolving action of some organic solvents over some of the membrane constituents.

These factors will account for the effects of heavy metal salts which at low concentrations act as coagulants, the effect of soaps, saponin, bile salts, etc., which are good peptising

[1] Mikwa: Biochem. Zeitschr., *149*, 550, 1924.

agent for lipoids, the action of narcotics and of hypotonic and hypertonic solution. The effect of acid and alkali already studied may also be due to a decrease or increase in the peptisability of the membrane or due to a hydrolytic effect on the lipoids. For a full discussion of this point our previous Paper[1] may be consulted.

There are however several minor points which cannot be clearly explained as yet. Thus it was suggested by us previously that the effect of normal serum in the retardation of hemolysis by different hemolytes is due to a displacement of adsorption of the hemolyte on the corpuscle surface, the serum being preferentially more adsorbed. This view agrees well with the experimental results of sedimentation and agglutination of corpuscles in presence of serum. The accelerating action of normal serum in some conditions as given in this Paper cannot however be explained so easily. We do not, however, believe that a new body of protein hemolyte complex having a much greater hemolytic efficiency is formed when serum is added to a taurocholate + corpuscles or oleate + corpuscle mixtures. The explanation of the acceleration observed when serum is added to a mixture of oleate and corpuscles or taurocholate and corpuscles must be due to a change in some condition of the experiment such as a change in the hydrogen ion concentration of the solution[2] or to a change in the physical condition of the protein. Under the experimental condition it is not likely that a completely new hemolysin of protein and hemolyte mixture may be formed when the serum is added only after addition of the hemolyte to the red cells, and that this body cannot be formed when serum is mixed with the hemolyte and the whole thing added to the corpuscles because no acceleration can be observed in the latter case also.

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[1] Sen and Basu : loc. cit.
[2] Compare F. Sachs : loc. cit.

SUMMARY

1. An experimental study has been made of the hemolysis of sheep's red blood corpuscles under different conditions.

2. It has been found that at comparatively higher concentrations of the cells and hemolyte sodium taurocholate gives an abnormal time-dilution curve; but at lower concentrations, the time-dilution curve is of much simpler nature. It is also shown that depending upon the conditions of the experiments sodium glycocholate may have either a higher or a lower hemolytic power than sodium taurocholate.

3. The effects of mixture of hemolytes have been studied. It has been found that mixtures like saponin and taurocholate, saponin and oleate, and oleate and taurocholate show an additive effect and no inhibition on the hemolytic powers of the constituents is observed.

4. Traces of acids accelerate markedly the action of saponin and taurocholate but retard that of oleate. Traces of alkali retard the action of saponin and taurocholate but accelerate that of oleate when the latter is in high concentration but retard when the oleate concentration is comparatively lower.

5. The effect of normal sheep serum on the hemolysis in the presence of other chemical hemolysers has been studied in detail. It has been shown that when serum is added to the red cells beforehand or when a mixture of serum and hemolyte is added to the corpuscles, there is always a retarding action of serum on hemolysis; but when serum is added after the addition of the hemolyte to the corpuscles, there may be either an inhibition or an acceleration of hemolysis depending on the particular concentration of the red blood cells, the hemolyte, the amount of serum added and in certain cases, on the time-interval after which this serum is added to the mixture of corpuscles and hemolyte. This has been found true only in the case of taurocholate and oleate as the hemolyte but saponin has not yet been found to give similar results. Experiments are now being made with other hemolytes to find out whether a similar acceleration of hemolysis by the addition of serum can be obtained or not.

6. The effect of human serum which normally contains an hemolysin for sheep corpuscles has been studied in the presence of chemical hemolysers. It has been found that in small concentration hemolytic serum also inhibits the hemolytic action of saponin, oleate, taurocholate, acid and alkali. The behaviour of hemolytic human serum at low concentration is qualitatively the same as that of normal sheep serum, but in higher concentration it has itself a hemolytic action even in presence of other chemical hemolysers and in this respect differs from normal sheep serum. When the complement was destroyed by heating the hemolytic serum at 56° C for half an hour, this complement-free human serum had a greater retarding action on hemolysis than the pure hemolytic serum.

7. A detailed study of hemolysis in sucrose solution has been made. It is observed that sucrose has an inhibiting effect on saponin, taurocholate and oleate hemolysis and its action is mainly on the corpuscles themselves. In all important respects, the results obtained in sucrose solution are quite similar to that obtained in saline solution.

8. The mechanism of hemolysis of blood corpuscles by hemolytes has been discussed. It is found that the membrane is in the colloidal state and hemolysis may occur either due to coagulation whereby cracks will be formed or due to peptisation of lipoids whereby these particles will go into apparent solutions thereby loosening the whole structure. In either case the permeability of the membrane will be greatly increased. In some cases, it may be due to a pure osmotic effect where a mechanical rupture of the membrane is possible. The effect of narcotics and organic solvents may be a peptisation phenomenon or a pure solubility effect and there may also be a hydrolytic effect in presence of acids and in presence of bacterial lysins.

SLOW AND INDUCED OXIDATION OF GLYCOGEN, LECITHIN, CHOLESTEROL, FORMATE, OLEATE, STEARATE AND SOME FOOD MATERIALS

BY

C. C. PALIT

In previous publications[1] we have shown that carbohydrates, fats and nitrogenous substances occurring either singly or in mixtures can be oxidised at the ordinary temperature by passing air in presence of reducing agents and some metallic hydroxides. We have also shown that the amount of oxidation of carbohydrates is greatly decreased by the presence of fats.

In this communication the results on the oxidation of glycogen, sodium formate, butter, cholesterol, lecithin, milk, egg-white and egg-yellow by air at the ordinary temperature occurring either singly or in mixtures in presence of different hydroxides or NaOH or $NaHCO_3$ or Na_2SO_3 are shown and we are also recording our experiments showing that the induced oxidations of potassium stearate and potassium oleate are retarded by the presence of different carbohydrates. In presence of carbohydrates and urea, the oxidations of potassium stearate and oleate are more retarded than in presence of carbohydrates alone.

[1] J. Phys. Chem., *29*, 376, 799 (1925); *30*, 939 (1926); *32*, 1663 (1928).

The experimental procedure is the same as described in previous papers and the temperature of the experiment is 25°. The results are as follows :—

TABLE I

Oxidation of Glycogen in Presence of Different Inductors

Estimation of glycogen in presence of (i) freshly precipitated hydroxides of different metals in neutral solutions, and (ii) caustic soda, sodium bicarbonate and sodium sulphite. The volume of glycogen used was 10 c.c. and the volumes of different salt solutions, each of 1% concentration, were 20 c.c. in each case. The hydroxides were precipitated from the salt solutions by the addition of exact equivalent amount of caustic soda. The volume of air passed was 36·5 litres in 5½ hours (10 c.c. of glycogen = 0·2343 grm., of CuO = 0·1065 grm. of glucose × 0·927 = 0·0987 grm. of glycogen).

Substance (freshly precipitated hydroxides, caustic soda, sodium bicarbonate, fats, or sodium sulphite) used in the experiment as catalyst.	Actual amount of copper oxide formed in grm. in 10 c.c. of the solution taken (Blank).	Amount of copper oxide formed in grm. after the experiment.	Amount of substance oxidised in grm. in terms of copper oxide.	Percentage amount of substance oxidised.
(1) Ferrous hydroxide ...	0·2343	0·0831	0·1512	64·5
(2) Cerous hydroxide ...	,,	0·0293	0·2050	87·5
(3) Manganous hydroxide ...	,,	0·0501	0·1842	78·6
(4) Uranous hydroxide ...	,,	0·0433	0·1910	81·5
(5) Cobaltous hydroxide ...	,,	0·0261	0·2082	88·9
(6) Nickelous hydroxide ...	,,	0·0253	0·2090	89·2
(7) Mercuric hydroxide (Wet HgO)	,,	0·1585	0·0758	32·35
(8) Chromic hydroxide ...	,,	0·1095	0·1248	53·3
(9) Ferric hydroxide ...	,,	0·0753	0·1590	67·9
(10) Cupric hydroxide ...	,,	0·0707	0·1636	69·8
(11) Caustic soda (=10 c.c. of N/8)	,,	0·1469	0·0874	37·3
(12) Sodium bicarbonate (=10 c.c. of 1% solution) ...	,,	0·2061	0·0282	12·0
(13) Sodium sulphite (=10 c.c. of 1% solution) ...	,,	0·1832	0·0511	21·8
(14) Potassium stearate (=10 c.c. of 1% solution) ...	,,	0·2241	0·0101	4·3
(15) Potassium oleate (=10 c.c. of 1% solution) ...	,,	0·2161	0·0182	7·8

TABLE II

Oxidation of Glycogen in Mixtures

Inductor—Ferrous Hydroxide

Estimation of glycogen in presence of freshly precipitated ferrous hydroxide (=0·0674 grm.) with (i) potassium stearate, (ii) potassium oleate, (iii) glycine, (iv) urea, and (v) glucose, in neutral solutions. The volume of each of the solutions taken was 10 c.c. of 1% concentration. The volume of air passed was 36·5 litres in $5\frac{1}{2}$ hours.

Substance used in the experiment as retarder.	Actual amount of copper oxide formed in grm. in 10 c.c. of the solution taken (Blank).	Amount of copper oxide formed in grm. after the experiment.	Amount of substance oxidised in grm. in terms of copper oxide.	Percentage amount of substance oxidised
(1) Potassium stearate ...	0·2343	0·1103	0·1240	52·9
(2) Potassium oleate ...	,,	0·1047	0·1296	55·3
(3) Glycine	,,	0·1358	0·0985	42·0
(4) Urea	,,	0·1442	0·0901	38·45
(5) Glucose	,,	0·1075	0·1268	54·1

Table III

Experiments with sodium formate in presence of (i) caustic soda, (ii) sodium bicarbonate, (iii) freshly precipitated ferrous hydroxide, and (iv) freshly precipitated cerous hydroxide. The volume of air passed was 36·5 litres in $5\frac{1}{2}$ hours.

Substance used as inductor in the experiment.	Amount of inductor in grm.	Actual amount of sodium formate in 10 c. c. of the solution taken before the expt. in grms. (Blank).	Amount of sodium formate left after the expt. in grm.	Amount of sodium formate oxidised in grm.	Percentage amount of substance oxidised.
(1) Caustic Soda ...	0·04	0·0737	0·0606	0·0131	17·8
(2) Sodium bicarbonate	0·1	,,	0·0656	0·0081	11·0
(3) Ferrous hydroxide	0·0648	,,	0·0576	0·0161	21·8
(4) Cerous hydroxide...	0·1069	,,	0·0565	0·0172	23·3

It appears from the above table that an aqueous solution of sodium formate can be oxidised by merely passing air at 25° in presence of sodium hydroxide, sodium bicarbonate, ferrous or cerous hydroxide.

TABLE IV

Oxidation of Potassium Stearate

Inductor—Ferrous hydroxide (=0·06476 grm.)

Estimation of potassium stearate in presence of freshly precipitated hydroxide with (*i*) carbohydrates, and (*ii*) carbohydrates+urea in neutral solution. The volume of air passed was 60 litres in 9 hours. The solutions of carbohydrates, urea and fat were each of 1% concentration.

Substance used in the experiment.	Amount of absorption of ICl_3 by fat in terms of N/10 $Na_2S_2O_3$ before expt.(Blank).	Oxidation in presence of carbohydrates.		Oxidation in presence of carbohydrates+urea.	
		Amount of absorption of ICl_3 by fat left in terms of N/10 $Na_2S_2O_3$ after the experiment.	Percentage amount of fat oxidised.	Amount of absorption of ICl_3 by fat left in terms of N/10 $Na_2S_2O_3$ after the experiment.	Percentage amount of fat oxidised.
	(1)	(2)	(3)	(4)	(5)
(1) Arabinose	5·8 c. c.	2·4 c. c.	58·6	2·5	56·9
(2) Galactose	„	2·5	56·9	2·6	55·2
(3) Glucose	„	2·8	51·7	3·4	41·3
(4) Laevulose	„	2·9	50·0	3·2	44·8
(5) Lactose	„	3·0	48·2	3·5	39·5
(6) Cane Sugar	„	2·7	53·4	3·5	39·5
(7) Maltose	„	2·9	50·0	3·3	43·1
(8) Starch	„	2·4	58·6	3·3	43·1

The amount of oxidation of potassium stearate by air with ferrous hydroxide as inductor and in absence of carbohydrates=63·8%.

The figures in columns 2 and 4 indicate the amount of absorption of ICl_3 by fat left after deducting the absorption

of ICl_3 by the respective carbohydrates and urea left in the mixture for which the absorptions were separately made.

TABLE V

Oxidation of Potassium Stearate

Inductor—Cerous hydroxide (=0·1069 grm.)

Estimation of potassium stearate in presence of freshly precipitated cerous hydroxide with (*i*) carbohydrates, and (*ii*) carbohydrates+urea in neutral solution. The volume of air passed was 60 litres in 9 hours. The solutions of carbohydrates, fat and urea were each of 1 % concentration.

Substance used in the experiment.	Amount of absorption of ICl_3 by fat in terms of N/10 $Na_2S_2O_3$ before expt. (Blank).	Oxidation in presence of carbohydrates.		Oxidation in presence of carbohydrates and urea.	
		Amount of absorption of ICl_3 by fat in terms of N/10 $Na_2S_2O_3$ left after the expt.	Percentage amount of substance oxidised.	Amount of absorption of ICl_3 by fat in terms of N/10 $Na_2S_2O_3$ left after the experiment.	Percentage amount of substance oxidised.
	(1)	(2)	(3)	(4)	(5)
(1) Arabinose	5·8	2·0	65·5	2·2	62·0
(2) Galactose	„	2·45	57·7	2·4	58·6
(3) Glucose	„	2·6	55·2	3·5	39·6
(4) Laevulose	„	2·85	50·9	3·2	44·8
(5) Lactose	„	2·9	50·0	3·4	41·4
(6) Cane Sugar	„	2·7	53·4	3·7	36·2
(7) Maltose	„	2·8	51·7	3·4	41·4
(8) Starch	„	2·5	60·3	3·2	44·8

The amount of oxidation of potassium stearate by air with cerous hydroxide as inductor and in absence of carbohydrates = 68·9 %.

The figures in columns 2 and 4 indicate the absorption of ICl_3 by fat left after deducting the absorption of ICl_3 by the respective carbohydrates and urea left in the mixture for which the absorptions were separately made.

TABLE VI

Oxidation of Potassium Oleate

Inductor—Ferrous hydroxide (=0·06476 grm.)

Estimation of potassium oleate in presence of freshly precipitated ferrous hydroxide with (i) carbohydrates, and (ii) carbohydrates+urea in neutral solution. The volume of air passed was 60 litres in 9 hours. The solutions of carbohydrates, fat and urea were each of 1% concentration.

Substance used in the experiment.	Amount of absorption of ICl_3 by fat in terms of N/10 $Na_2S_2O_3$ before expt. (Blank).	Oxidation of fat in presence of carbohydrates.		Oxidation of fat in presence of carbohydrates and urea.	
		Amount of absorption of ICl_3 by fat in terms of N/10 $Na_2S_2O_3$ left after the expt.	Percentage amount of substance oxidised.	Amount of absorption of ICl_3 by fat in terms of N/10 $Na_2S_2O_3$ left after the expt.	Percentage amount of substance oxidised.
	(1)	(2)	(3)	(4)	(5)
(1) Arabinose ...	6·6	3·6	45·4	3·9	40·6
(2) Galactose ...	,,	3·95	41·7	4·05	38·6
(3) Glucose ...	,,	4·05	38·6	4·5	31·8
(4) Laevulose ...	,,	4·1	38·0	4·2	36·3
(5) Lactose ...	,,	4·3	34·8	4·6	30·3
(6) Cane Sugar ...	,,	3·9	40·9	4·6	30·3
(7) Maltose ...	,,	4·2	36·3	4·4	33·3
(8) Starch ...	,,	3·7	43·9	4·4	33·3

The amount of oxidation of potassium oleate by air with ferrous hydroxide as inductor and in absence of carbohydrates = 47%.

The figures in columns 2 and 4 indicate the absorption of ICl_3 by fat left, after deducting the absorption of ICl_3 by the respective carbohydrates and urea left in the mixture for which the absorptions were separately made.

We have also carried on some experiments on the oxidation of carbohydrates, fats and nitrogenous substances either occurring singly or in mixtures by hydrogen peroxide and ferric sulphate. The results are as follows :—

TABLE VII (*a*)

Experiments on oxidation of carbohydrates, nitrogenous substances and fats by hydrogen peroxide in presence of ferric sulphate at 50°. The volume of each of the substances under investigation was 10 c.c. of M/10 concentration, where M represents a molecule of the substance. The volume of ferric sulphate taken was 1 c.c. (=0·00158 grm. of Fe_2O_3). Time of oxidation was 2 hours in each case.

Substance used in the experiment.	Percentage amount of oxidation of substances occurring singly or in presence of						
	Singly	Glycine.	Potassium oleate.	Potassium stearate.	Potassium palmitate.	Glycine + pot. oleate.	Glycine + potassium stearate.
(1) Glucose ...	78·3	70·0	12·5	33·0	23·3	29·4	25·5
(2) Starch ...	69·6	59·5	27·7	35·8	...	44·4	54·3
(3) Cane Sugar ...	90·0	...	...	...	24·8	...	...
(4) Maltose ..	93·0	...	...	...	86·5	...	...
(5) Lactose ...	92·4	...	...	...	84·5	...	...

TABLE VII (*b*)

Substance used in the experiment.	Percentage amount of oxidation of substances occurring singly or in presence of				
	Singly.	Glucose.	Starch.	Glucose + potassium oleate.	Glucose + glycine.
(1) Glycine ...	32·8	17·2	20·0	15·4	...
(2) Potassium oleate	70·7	30·9	32·1	...	28·6

The foregoing results prove that the oxidations of glucose, starch, canesugar, maltose and lactose by H_2O_2 and ferric salts are retarded by glycine, potassium oleate and potassium palmitate.

Similarly the oxidations of glycine and potassium oleate by H_2O_2 and ferric sulphate are retarded by glucose, starch, etc. The retardation of the oxidation of carbohydrates is more pronounced in presence of fats than glycine.

In order to find out whether the carbohydrates, fats and nitrogenous substances are oxidised completely to carbon dioxide or other intermediate products were formed, we have estimated the amount of carbon dioxide obtained in these oxidations in potash bulbs. The amount of oxidation of these substances was in all cases also estimated by direct analysis as in previous cases. The experimental results are recorded below:

TABLE XIII

Inductor—Fe $(OH)_2$

Experiments on the oxidation of carbohydrates, proteins and fats, in presence of freshly precipitated ferrous hydroxide

and the estimation of carbon dioxide formed. The volume of air passed was 75 litres in 11 hours. The amount of ferrous hydroxide used was 0·06476 grm.

Substance used in the experiment.	Amount of substance taken in 10 c.c. of the solution before experiment (Blank).	Amount of CO_2 formed as found by experiment.	Amount of substance oxidised as calculated from the value of CO_2 found.	Percentage amount of substance oxidised.	Amount of substance oxidised as found by direct estimation.	Percentage amount of substance oxidised.
(1) Glucose	0·1255 grm.	0·0529	0·0369	36·9	0·0427	24·0
(2) Glycine	0·0915 „	0·0217	0·0185	18·5	0·0180	19·6
(3) Potassium oleate	5·5 c.c. (= 0·1 grm.)	0·0224	0·0091	9·1*	0·6 c.c.	10·9
(4) Lecithin	5·0 c.c. (= 0·1 grm.)	0·0206	0·0086	8·6	0·45 c.c.	9·0
(5) Cholesterol	1·35 c.c. (= 0·035 grm.)	0·0182	0·0059	16·9	0·20 c.c.	14·8
(6) Milk ...	10·75 c.c. ...	0·0203	...	...	1·65 c.c.	13·5
(7) Egg-yellow (5% solution)	6·7 c.c. (=0·5 grm.)	0·0134	...	...	3·70 c.c.	55·2
(8) Egg-white (1% solution)	0·75 c.c. (= 0·1 grm.)	0·0125	...	...	0·05 c.c.	6·7
(9) Egg-white (5% solution)	1·6 c.c. (= 0·5 grm.)	...	...	...	0·30 c.c.	18·75

N.B.—The figures in Nos. 3 to 5 represent the amount of absorption of ICl_3 by the fat in terms of N/10 $Na_2S_2O_3$ and in Nos. 6 to 9 the amount of absorption of ammonia by the acid as determined by Kjeldalh's method—in terms of N/10 NaOH before and after the experiments.

* In the case of potassium oleate, the amount of carbon dioxide obtained by experiment was a little lower than the calculated amount and this low value may be due to the absorption of CO_2 by the alkali set free by the hydrolysis of potassium oleate.

TABLE IX

Inductor—Ce $(OH)_3$

Experiments on the oxidation of carbohydrates, proteins and fats in presence of freshly precipitated cerous hydroxide and the estimation of carbon dioxide formed. The volume of air passed was 75 litres in 11 hours. The amount of cerous hydroxide used was 0·1069 grm.

Substance used in the experiment.	Amount of substance taken in 10 c.c. of the solution before experiment (Blank).	Amount of CO_2 formed as found by experiment.	Amount of substance oxidised as calculated from the value CO_2 found.	Percentage amount of substance oxidised.	Amount of substance oxidised as found by direct estimation.	Percentage amount of substance oxidised.
(1) Glucose ...	0·1034 grm. ...	0·0174	0·0120	12·0	0·0130	12·6
(2) Glycine ...	0·0900 ,, ...	0·0562	0·0479	47·9	0·04275	47·5
(3) Potassium oleate	5·5 c.c. N/10 $Na_2S_2O_3$ (0·1 grm.)	0·0184	0·0074	7·4*	0·55 c.c. N/10 $Na_2S_2O_3$	10·0
(4) Lecithin ...	5·0 c.c. N/10 $Na_2S_2O_3$ (0·1 grm.)	0·0186	0·0078	7·8	0·40 c.c. N/10 $Na_2S_2O_3$	8·0
(5) Cholesterol ...	1·35 c.c. N/10 $Na_2S_2O_3$ (0·035 grm.)	0·0153	0·0050	14·3	0·20 c.c. N/10 $Na_2S_2O_3$	14·8
(6) Milk ...	10·75 c.c. N/10 NaOH.	0·0221	...	...	1·45 c.c. N/10 NaOH	15·4
(7) Egg-yellow (5 % solution)	6·7 c.c. N/10 NaOH (0·5 grm.)	0·0189	...	...	3·8 c.c. N/10 NaOH	56·7
(8) Egg-white (1 % solution)	0·75 c.c. N/10 NaOH (0·1 grm.)	0·0378	...	...	complete ...	100·0
(9) Egg-white (5 % solution)	1·6 c.c. N/10 NaOH (0·5 grm.)	...	...	...	1·3 c.c. N/10 NaOH.	81·25

* In the case of potassium oleate, the amount of CO_2 obtained by experiment was a little lower than the calculated amount and this low value may be due to the absorption of CO_2 by the alkali set free by hydrolysis of potassium oleate.

TABLE X

Inductor—Na_2SO_3

Experiments on the oxidation of carbohydrates, proteins and fats in presence of sodium sulphite (=0·1513 grm.) and the estimation of carbon dioxide formed. The volume of air passed was 75 litres in 11 hours.

Substance in the experiment.	Amount of substance taken in 10 c. c. of the solution before experiment (Blank).	Amount of CO_2 formed as found by experiment.	Amount of substance oxidised as calculated from the value of CO_2 found.	Percentage amount of substance oxidised.	Amount of substance oxidised as found by direct estimation.	Percentage amount of substance oxidised.
(1) Glucose ...	0·0962 grm.	0·0406	0·0277	27·7*	0·0347	36·0
(2) Glycine ...	0·0915 grm.	0·0320	0·027	27·0	...	...
(3) Potassium oleate	6·5 c. c. (0·1 grm.)	0·0120	0·005	5·0*	0·45 c. c.	6·9
(4) Lecithin ...	5·0 c. c. (0·1 grm.)	0·0165	0·0069	6·9	0·35 c. c.	8·1
(5) Cholesterol ...	1·35 c. c. (0·35 grm.)	0·0442	0·0144	41·1	0·55 c. c.	40·8
(6) Milk ...	10·75 c. c.	0·01265	...	...	1·05 c. c.	9·7
(7) Egg-yellow (5 % solution)	6·7 c. c. (0·5 grm.)	0·112	...	...	3·85 c. c.	57·5
(8) Egg-white (1 % solution)	0·75 c. c. (0·1 grm.)	0·0291	...	...	0·15 c. c.	20·0
(9) Egg-white (5 % solution)	1·6 c. c. (0·5 grm.)	...	...	...	0·3 c. c.	18·75

N.B.—The figures in Nos. 3 to 5 represent the amount of absorption of ICl_3 by the fat in terms of N/10 $Na_2S_2O_3$ and in Nos. 6 to 9 the amount of absorption of ammonia by the acid—as determined by Kjeldalh's method—in terms of N/10 NaOH before and after the experiments.

*In the case of potassium oleate and glucose the amount of CO_2 as obtained by experiment was a little lower than the calculated amount and this low value may be due to the absorption of CO_2 by the alkali set free by the hydrolysis of potassium oleate and sodium sulphite.

We have tried to obtain quantitative and comparative results on the amount of oxidation of carbohydrates, fats and proteins by air and thus establish whether fats or carbohydrates or nitrogenous substances are more readily oxidised in the system or outside. The experimental results are as follows :—

Comparative experiments on the oxidation of carbohydrates, nitrogenous substances and fats in presence of (i) caustic soda, and (ii) sodium bicarbonate and in absence of any inductor.

TABLE XI

In presence of NaOH

In these experiments, the volume of each of the solutions taken was 10 c.c. of 1% concentration. The volume of air passed was 36·5 litres in 5½ hours and the amount of alkali used was 10 c.c. of N/10 NaOH (=0·04 grm.).

Substance used in the experiment.	Actual amount of substance in 10 c. c. of the solution (Blank).	Amount of substance left after the experiment.	Amount of substance oxidised.	Percentage amount of substance oxidised.
(1) Glucose ...	0·0962 grm.	0·0857 grm.	0·0105 grm.	10·9
(2) Starch ...	0·1027 ,,	0·0701 ,,	0·0326 ,,	31·7
(3) Alanine ...	0·0997 ,,	0·0730 ,,	0·0267 ,,	26·8
(4) Glycine ...	0·0999 ,,	0·0750 ,,	0·0249 ,,	24·9
(5) Potassium stearate*	0·55 c. c.	0·35 c. c.	0·20 c. c.	36·3
(6) Potassium oleate*	5·85 c. c.	4·35 c. c.	1·5 c. c.	26·5

* The figures indicate the amount of absorption of ICl_3 by fat in terms of N^1 10 $Na_2S_2O_3$, before and after the experiment.

Table XII

In presence of $NaHCO_3$

In these experiments, the volume of each of the solutions taken was 10 c.c. of 1% concentration. The volume of air passed was 36·5 litres in 5½ hours and the amount of bicarbonate used was 0·1 grm.

Substance used in the experiment.	Actual amount of substance in 10 c. c. of the solution (Blank).	Amount of substance left after the experiment.	Amount of substance oxidised.	Percentage amount of substance oxidised.
(1) Glucose ...	0·0962	0·0779	0·0183	19·0
(2) Starch ...	0·1027	0·0658	0·0367	35·9
(3) Alanine ...	0·0997	0·0703	0·0294	29·5
(4) Glycine ...	0·0999	0·0682	0·0317	31·5
(5) Potassium stearate*	0·55 c. c.	0·40 c. c.	0·15 c. c.	30·0
(6) Potassium oleate*	5·85 c. c.	5·2 c. c.	0·65 c. c.	11·2

Comparative experiments with carbohydrates, proteins and fats in presence of (i) freshly precipitated cerous hydroxide, (ii) sodium bicarbonate, and (iii) caustic soda.

Table XIII

In Presence of Cerous Hydroxide=(0·1069 grm.).

Estimation of carbohydrates, nitrogenous substances and

* The figures indicate the amount of absorption of ICl_3 by fat in terms of N/10 $Na_2S_2O_3$ before and after the experiment.

fat in presence of freshly precipitated cerous hydroxide. The volume of air passed was 60 litres in 9 hours.

Substance used in the experiment.	Amount of substance taken in 10 c. c. of the solution before experiment (Blank).	Amount of substance left after the experiment.	Amount of substance oxidised.	Percentage amount of substance oxidised.
(1) Egg-white (5% solution) ...	1·55 c. c.	0·25 c. c.	1·3 c. c.	83·3
(2) Egg-yellow (5% solution) ...	8·35 c. c.	2·55 c. c.	5·8 c. c.	69·4
(3) Starch ...	0·1027 grm.	0·0439 grm.	0·0588 grm.	57·2
(4) Glucose ...	0·1034 grm.	0·0657 grm.	0·0377 grm.	36·5
(5) Butter (= 0·9320 grm.)	12·7 c. c.	8·9 c. c.	3·8 c. c.	29·9

N.B.—The figures in Nos. 1 and 2 represent the amount of absorption of ammonia by the acid—as determined by Kjeldalh's Method—in terms of N/10 NaOH and in No. 5, the absorption of ICl_3 by butter in terms of N/10 $Na_2S_2O_3$.

TABLE XIV

In Presence of Sodium-bicarbonate (=0·10 grm.)

Estimation of carbohydrates, fat and nitrogenous substances in presence of sodium bicarbonate. The volume of air passed was 60 litres in 9 hours.

Substance used in the experiment.	Amount of substance taken in 10 c. c. of the solution before experiment (Blank).	Amount of substance left after the experiment.	Amount of substance oxidised.	Percentage amount of substance oxidised.
(1) Starch ...	0·1027 grm.	0·0699 grm.	0·0328 grm.	31·9
(2) Butter (= 0·932 grm.)	12·7 c. c.	9·4 c. c.	3·3 c. c.	26·0
(3) Glucose ...	0·1034 grm.	0·0896 grm.	0·0138 grm.	13·3
(4) Egg-yellow (=5% solution) ...	8·35 c. c.	7·35 c. c.	1·00 c. c.	12·0
(5) Egg-white (=5% solution) ...	1·55 c. c.	1·40 c. c.	0·15 c. c.	9·7

The figures in No. 2 represent the amount of absorption of ICl_3 by butter in terms of N/10 $Na_2S_2O_3$ and in Nos. 4 and 5 the amount of absorption of ammonia by the acid—as determined by Kjeldalh's method—in terms of N/10 NaOH.

The foregoing results show that in presence of sodium bicarbonate, the oxidation of egg-yellow and egg-white is much less than in presence of cerous hydroxide in neutral solution. It seems likely that the fat present in egg is partly separated and saponified and this fat retards the oxidation of the protein matter of egg.

TABLE XV

In Presence of Caustic-soda (0·04 grm.)

Estimation of carbohydrates, fat and nitrogenous substances in presence of caustic soda. The volume of air passed was 60 litres in 9 hours.

Substance used in the experiment.	Amount of substance taken in 10 c.c. of the solution before experiment (Blank).	Amount of substance left after the experiment.	Amount of substance oxidised.	Percentage amount of substance oxidised.
(1) Starch ...	0·1027 grm.	0·0661 grm.	0·0466 grm.	45·3
(2) Egg-yellow (=5 % solution) ...	8·35 c.c.	5·95 c.c.	2·4 c.c.	28·7
(3) Egg-white (=5 % solution) ...	1·55 c.c.	1·20 c.c.	0·35 c.c.	22·5
(4) Butter (= 0·923 grm.)	12·7 c.c.	10·2 c.c.	2·50 c.c.	19·7
(5) Glucose ...	0·1034 grm.	0·0839 grm.	0·0195 grm.	18·8

The figures in Nos. 2 and 3 represent the amount of absorption of ammonia by acid as determined by

Kjeldalh's method in terms of N/10 NaOH and in No. 4 the amount of absorption of ICl_3 by butter in terms of N/10 $Na_2S_2O_3$.

In this case also, the oxidation of the protein matter in eggs, appears to be retarded by the partially saponified fat also present in the eggs.

DISCUSSION

The experimental results recorded in the Table I show that glycogen can be oxidised by passing air at the ordinary temperature in presence of one of the following substances :—

$NaHCO_3$, NaOH, Na_2SO_3, $Fe(OH)_2$, $Ce(OH)_3$, $Mn(OH)_2$, $UO_2(OH)_2$, $Co(OH)_2$, $Ni(OH)_2$, HgO, $Cr(OH)_3$, $Fe(OH)_3$, $Cu(OH)_2$. From the results recorded in Table II, it will be observed that the amount of glycogen oxidised by air at the ordinary temperature with ferrous hydroxide as the inductor is decreased by potassium stearate, oleate, glycine, urea and glucose and all these latter substances are also simultaneously oxidised along with glycogen.

These results on the slow and induced oxidation of glycogen are interesting in view of the fact that glycogen, which is present in liver and muscle, is looked upon as reserve form of carbohydrate, which plays very much the same role in animal metabolism as starch does in plant life. Moreover glycogen stands in very close connection with the general questions of carbohydrate metabolism. The results recorded in Tables IV, V and VI show that the induced oxidation of potassium stearate and oleate by air at the ordinary temperature is appreciably retarded by arabinose, galactose, glucose, laevulose, lactose, cane sugar, maltose and starch. Moreover, in presence both of urea and carbohydrates, the oxidation of potassium stearate and oleate is more retarded than in presence of carbohydrates alone. These results are likely to throw considerable light on the

metabolism in the animal body in normal health and diabetes.

The function of the fats is, like that of the carbohydrates, to supply heat and energy. Within certain limits, fats and carbohydrates can be used alternatively in the diet. If all the carbohydrate in the food be replaced by fats, the fat is incompletely oxidised; in other words, carbohydrate burns more easily than fat and for complete combustion of fat in the body, carbohydrate must be burned along with it. Very little carbohydrate and a great deal of fat are eaten by the Eskimos but they eat an abnormal amount of protein; in this case the proteins act like carbohydrates in retarding the oxidation and assisting in the complete oxidation (combustion) of fat.

The foregoing results show that the amount of carbon-dioxide obtained in these slow oxidations is practically the same as is expected from the point of view that the carbohydrates, fats and nitrogenous substances are completely oxidised into carbon dioxide and water by passing air at the ordinary temperature. We venture to think that these results are of importance because these oxidations are of the same type as those taking place in the animal body.

The oxidation of organic substances by hydrogen peroxide and iron salts has been investigated by Dakin and co-workers and others, but in these cases several complex intermediate products are obtained and these slow oxidations appear different from those taking place in the animal body. As far as we know we have been the first to prove conclusively in a systematic manner that fats, carbohydrates, nitrogenous and other organic substances can be completely oxidised into their main end products, carbon dioxide and water by air with the help of an inductor or in presence of light at the ordinary temperature and have thus been able to imitate successfully the physiological processes of oxidation on which animal life depends.

Voit stated: "That the metabolism in the body was not proportional to the combustibility of the substances outside the body, but that protein which burns with difficulty outside metabolises with the greatest ease, then carbohydrates, while fat which readily burns outside is the most difficultly combustible in the body."

This conclusion was arrived at by Voit from actual feeding experiments on animals.

We have obtained quantitative and comparative results on the velocity of oxidation of fats, proteins, and carbohydrates by air and thus tried to establish whether fats or carbohydrates are oxidised more readily in the system.

Our experimental results (*vide* Table XIII) show that the order in which they are oxidised in presence of cerous hydroxide is as follows:—

Egg-white > egg-yellow > starch > glucose > butter; whereas the orders are different in presence of sodium bicarbonate or caustic soda. In presence of cerous hydroxide, the induced oxidation of fats, nitrogenous substances and carbohydrates follows the same order as stated by Voit.

Further work in this line is in progress in these laboratories.

SUMMARY

1. An aqueous solution of sodium formate and an aqueous suspension of glycogen has been oxidised by air at 25° in presence of NaOH, $NaHCO_3$ and different metallic hydroxides.

2. The oxidation of glycogen is retarded by fats and nitrogenous substances.

3. The induced oxidation of potassium stearate and oleate by air is retarded by carbohydrates and to a greater extent by a mixture of carbohydrates and urea.

4. The oxidation of carbohydrates by H_2O_2 and ferric-sulphate is retarded by glycine, potassium stearate and oleate.

5. Experimental results on the estimation of carbon dioxide prove that glucose, glycine, potassium oleate, lecithin, cholesterol, milk, egg-yellow and egg-white are oxidised by air at 25° in presence of inductors chiefly to carbon dioxide and not to any intermediate product.

6. Comparative experiments have been carried on the induced oxidation of fats, carbohydrates and nitrogenous substances to prove whether nitrogenous matter is more readily oxidised than carbohydrates. The results show that in neutral solutions with cerous hydroxide as an inductor, the order in which the oxidation takes place is as follows :—Egg-white > egg-yellow > starch > glucose > butter. This order is the same as stated by Voit, the eminent physiologist, from physiological experiments.

7. These results on slow and induced oxidation of fats, nitrogenous substances and carbohydrates occurring either singly or in mixtures by air at the ordinary temperature are important, because these oxidations are of the same type as those taking place in the animal body.

PHOTO-CHEMICAL OXIDATION OF SALTS OF SOME ORGANIC ACIDS, ORGANIC ACIDS, LECITHIN, CHOLESTEROL AND SOME FOOD MATERIALS BY AIR

BY

C. C. PALIT.

In previous papers[1] we have shown that in presence of light several carbohydrates, glycogen, nitrogenous substances, and potassium palmitate, oleate and stearate and oxalate can be oxidised by simply passing air at the ordinary temperature. We have also shown that in presence of zinc oxide the amount of oxidation of these substances is increased.

We have carried on further experiments in this line and have been able to oxidise sodium tartrate, formate, citric acid, lactic acid, oxalic acid, tartaric acid, lecithin cholesterol, butter, milk, egg-white, and egg-yellow by passing air in presence of sunlight. Moreover we have investigated several of these photochemical oxidations in presence of ferric nitrate and uranium nitrate as photocatalysts.

In order to find out whether in presence of light the carbohydrates, fats and nitrogenous substances are oxidised completely to carbon dioxide or other intermediate products are formed, we have estimated the amount of carbon dioxide obtained in these oxidations in potash bulbs. The amount of photochemical oxidation of these substances was in all cases also estimated by direct analysis.

We have also studied whether the Stark-Einstein Law of photochemical equivalence is applicable to the photo-oxidation of carbohydrates and nitrogenous substances. The experimental arrangement is the same as that described in a previous paper. The experimental results are recorded below :

[1] J. Phys. Chem. *29*, 926 (1925); *32*, 1263 (1928).

TABLE I

In each of these experiments, the volume of air passed was 36·5 litres in $5\frac{1}{2}$ hours.

The tartrate was estimated as potassium bitartrate and formate by the precipitation of mercurous chloride.

No. of experiments.	Substance used in the experiment.	Actual weight of substance in 10 c.c. of the solution taken in grm. (Blank).	Amount of substance oxidised in grm	Percentage amount of substance oxidised.
1	Sodium tartrate ...	0·0989	0·0679	31·3
2	Sodium formate ...	0·07373	0·01467	19·9

The foregoing results prove that solutions of sodium tartrate and formate can be oxidised in presence of sunlight by passing air.

In recent papers[1] from this laboratory, it has been proved that zinc oxide is a powerful photochemical sensitiser and many photochemical reactions have been accelerated by the presence of zinc oxide. We have carried on experiments on the oxidation of sodium tartrate and sodium formate in sunlight in presence of zinc oxide as photosensitiser. The results are as follows:

TABLE II

In each of these experiments, the volume of air passed was 36·5 litres in $5\frac{1}{2}$ hours. The weight of zinc oxide taken was exactly 0·5 grm.

No. of experiments.	Substance used in the experiment.	Actual amount of substance in 10 c.c. of the solution taken in grm. (Blank).	Amount of substance oxidised in grm.	Percentage amount of substance oxidised.
1	Sodium tartrate ...	0·0989	0·0349	64·7
2	Sodium formate ...	0·07373	0·02703	35·3

[1] J. Ind. Chem. Soc., *4*, 299 (1927) ; J. Phys. Chem., *32*, 1263 (1928).

The foregoing experimental results show that the amount of oxidation of sodium tartrate and formate is greater in presence of zinc oxide, which acts as a photo-sensitiser. Recently it has been shown in this laboratory[1] that ferric chloride and uranium nitrate markedly increase the absorption of incident radiation and hence act as accelerators of photochemical reactions. We have carried on experiments on the photochemical oxidation of carbohydrates, fats and nitrogenous and other substances in presence of ferric nitrate and uranium nitrate. The results are as follows :

TABLE III

Photochemical oxidation of carbohydrates, nitrogenous products, fats and other substances in presence of ferric nitrate in sunlight.

In each of these experiments, the volume of air passed was 36·5 litres in 5½ hours. The amount of ferric nitrate taken was exactly 0·5 grm. in each case.

Substance used in the experiment.	Actual amount of substance in 10 c.c. of the solution taken in grm. (Blank). (1)	Amount of substance left after the experiment in grm. (2)	Amount of substance oxidised in grm. (3)	Percentage amount of substance oxidised. (4)	Percentage oxidised in absence of catalyst. (5)
1. Glucose ...	0.0962	0·0084	0·0878	91·3	14·9
2. Starch ...	0·1027	0·0072	0·0955	92·9	38·8
3. Glycogen ...	0·0987	0·0174	0·0813	82·3	19·7
4. Glycine ...	0·0999	0·02625	0·0735	73·7	9·6
5. α-Alanine ...	0·0997	0·0116	0·0881	88·3	36·6
6. Hippuric acid ...	0·0701	0·01845	0·05165	73·65	14·2
7. Urea ...	0·2000	0·1800	0·0200	10·0	8·7
8. Sodium urate ...	0·0375	0·0090	0·0285	76·0	19·6
9. Sodium formate	0·7373	*Nil*	complete.	100·0	19·9
10. Sodium tartrate	0·0989	,,	,,	,,	31·3
11. Potas. stearate*	1·4	0·7	0·7	50·0	40·0
12. Potas. oleate*	5·8	3·45	2·35	40·5	31·5

* These figures indicate the amount of absorption of ICl_3 in terms of N/10 $Na_2S_2O_3$ before and after the experiment and hence the amount of oxidation was determined.

[1] J. Ind. Chem. Soc., *5*, 411 (1928).

TABLE IV

Photochemical oxidation of different substances in presence of uranium nitrate.

In each of these experiments, the volume of air passed was 36·5 litres in 5½ hours. The amount of uranium nitrate taken was exactly 0·5 grm.

Substance used in the experiment.	Actual amount of substance taken in 10 c. c. of the solution in grm. (Blank).	Amount of substance left in grm. after experiment.	Amount of substance oxidised in grm.	Percentage amount of substance oxidised.
1. Glucose	0·0962	*Nil*	Complete	100·0
2. Starch	0·1027	,,	,,	,,
3. Glycogen	0·0987	0·0678	0·1665	71·0
4. Glycine	0·0999	0·0401	0·0598	59·75
5. α-Alanine	0·0632	0·0098	0·0534	84·5
6. Hippuric acid ...	0·0680	0·0358	0·0322	47·35
7. Sodium urate ...	0·0247	0·0116	0·0131	53·0
8. Potas. oxalate ...	0·1030	0·0129	0·0901	87·5
9. Sodium formate ...	0·07373	*Nil*	Complete	100·0
10. Sodium tartrate ..	0·0939	,,	,,	100·0
11. Potas. stearate* ...	0·75	0·30	0·45	60·0
12. Potas. oleate* ...	5·8	2·4	3·4	58·6

The experimental results recorded in the two foregoing papers show that the oxidation of glucose, starch, glycogen, glycine, α-alanine, hippuric acid, urea, sodium urate, sodium

* These figures indicate the amount of absorption of ICl_3 in terms of N/10 $Na_2S_2O_3$ before and after the experiment and hence the amount of oxidation was determined.

formate, sodium tartrate, potassium stearate and potassium oleate by air is greatly increased in presence of ferric nitrate and uranium nitrate, which act as photocatalysts.

TABLE V

Experiments with aqueous solution of organic acids in presence of sunlight.

The volume of air passed was 36·5 litres in $5\frac{1}{2}$ hours and the volume of acid solutions taken was 10 c. c. of 1% concentration.

Substance used in the experiment.	Actual amount of acid taken in 10 c. c. of the solution in terms of N/10 NaOH in c.c. (Blank).	Amount of acid left after the experiment in terms of N/10 NaOH in c.c.	Amount of acid oxidised in terms of N/10 NaOH in c.c.	Percentage amount of the substance oxidised.
1. Citric acid	14·3	14·05	0·25	1·75
2. Lactic acid	8·35	8·05	0·30	3·6
3. Oxalic acid	15·6	13·15	2·45	15·7
4. Tartaric acid ...	13·25	13·0	0·25	1·9

The above results show that the organic acids can be oxidised by air in presence of sunlight at the ordinary temperature. The order in which they are oxidised is the following :—

Oxalic acid > lactic acid > tartaric acid > citric acid.

In order to find out whether in presence of sunlight the carbohydrates, fats, and nitrogenous substances are oxidised completely to carbon dioxide or other intermediate products are formed, we have estimated the amount of carbon dioxide obtained in these oxidations in potash bulbs. The amount of oxidation of these substances was in all cases also estimated by direct analysis as in previous cases. The results are as follows :

TABLE VI

Estimation of Carbon dioxide

Experiments on the oxidation of carbohydrates, fats and nitrogenous substances in sunlight and their estimation of carbon dioxide.

Substance used in the experiment.	Amount of substance taken in 10 c.c. of the solution (Blank). (a)	Time of exposure in sunlight in hours. (b)	Amount of CO_2 as found by experiment. (c)	Amount of substance oxidised as calculated from the value of CO_2 found. (d)	Percentage amount of substance oxidised. (e)	Amount of substance oxidised as found by direct estimation. (f)	Percentage amount of substance oxidised. (g)
1. Glucose	0·1249	15	0·0144	0·0099	9·9	0·0131	10·4
2. Glycine	0·0999	11	0·0365	0·0311	31·1	0·0315	31·5
3. Potassium oleate ...	4·8 c.c.	9	0·0206	0·0083	8·3*	4·35 c.c.	9·3
4. Lecithin (=0·113 grm.) ...	4·4 c.c.	13	0·1196	0·0500	50·0	2·3 c.c.	52·3
5. Cholesterol (=0·035 grm.)...	1·1 c.c.	$5\frac{1}{2}$	0·0192	0·0062	17·8	0·2 c.c. (=·0064 grm.)	18·2
6. Butter (=1·0942 grm.) ...	5·7 c.c.	16	0·0471	...	...	3·15 c.c.	55·3
7. Milk	10·24 c.c.	$5\frac{1}{2}$	...	...	...	1·4 c.c.	13·7
8. Egg-white (1% solution) ...	0·75 c.c.	,,	...	...	...	0·15 c.c.	20·0
9. Egg-yellow (5% solution) ...	6·7 c.c.	,,	...	...	...	3·8 c.c.	56·7

N.B.—The figures from 3 to 9 in columns (*a*) and (*f*) indicate the amount of absorption of ICl_3 by the substances in terms of N/10 $Na_2S_2O_3$ before and after the experiment and the figures in 8 and 9 in the same columns indicate the amount of absorption of ammonia by the acid in term of N/10 NaOH as determined by Kjeldalh's method before and after the experiment.

* In the case of potassium oleate, the amount of carbon dioxide obtained by experiment was a little lower than the calculated amount and this low value may be due to the absorption of CO_2 by the alkali set free by the hydrolysis of potassium oleate.

TABLE VII

Experiments on the oxidation of different substances in sunlight by air in open vessels of different superficial area.

In each of these experiments, the volume of air passed was 36·5 litres in 5½ hours.

Substance used in the experiment.	Large surface. Area of the surface = 54·13 sq. cm.				Small surface. Area of the surface = 35·27 sq. cm.			
	Actual amount of the substance taken in 10 c. c. of the solution in grm. before experiment (Blank.)	Amount of substance left in grm. after the experiment.	Amount of substance oxidised in grm.	Percentage amount of substance oxidised.	Actual amount of substance taken in 10 c. c. of the solution in grm. before experiment (Blank).	Amount of substance left in grm. after the experiment.	Amount of substance oxidised in grm.	Percentage amount of substance oxidised.
1. Glucose ...	0·1125	0·0974	0·0151	13·4	0·1125	0·1038	0·0087	7·7
2. Glycine ...	0·09825	0·0750	0·02325	23·7	0·09825	0·08925	0·0090	9·2
3. Alanine ...	0·0845	0·0534	0·0311	36·8	0·0845	0·0645	0·0200	23·7
4. Lactose ...	0·0881	0·0681	0·0200	22·7	0·0881	0·0764	0·0117	13·3
5. Butter (=1.0892 grm.) ..	5·66 c. c.	5·1 c. c.	0·56 c. c.	9·9	5·64 (=1·0840 grm.)	5·2 c. c.	0·44 c. c.	7·8
6. Milk ...	10·2 c. c.	7·4 c. c.	2·8 c. c.	27·5	10·2 c. c	8·7 c. c.	1·5 c. c.	14·7
7. Egg-white (5% solution)	1·6 c. c.	1·1 c. c.	0·5 c. c.	31·25	1·6 c. c.	1·2 c. c.	0·4 c. c.	25·0
8. Egg-yellow (5% solution)	6·7 c. c.	1·75 c. c.	4·95 c. c.	73·9	6·7 c. c.	2·8 c. c.	3·9 c. c.	58·2

N. B.—The figures in 5 and 6 indicate the amount of absorption of ICl_3 by the substance in terms of N/10 $Na_2S_2O_3$ before and after the experiment and figures in 7 and 8 indicate the amount of absorption of ammonia by the acid in term of N/10 NaOH as determined by Kjeldalh's method before and after the experiment.

The foregoing results show that the amount of oxidation increases with the amount of light falling on the solutions.

TABLE VIII

Experiments on the oxidation of (i) glucose, (ii) glycine, and (iii) potassium oleate by air in sunlight with respect to the time of exposure. (10 c. c. of glycine = 15·6 c. c. of N/12 NaOH and the absorption of ICl_3 by 10 c. c. of potassium oleate in terms of thiosulphate = 5·4 c. c. N/10 $Na_2S_2O_3$).

Substance used in the experiment.	Actual amount of the substance taken in 10 c. c. of the solution before the experiment (Blank).	Length of exposure in hours.	Amount of substance left after the experiment.	Amount of substance oxidised.	Percentage of substance oxidised.
(A) Glucose	0·1062 grm.	4	0·1004 grm.	0·0058 grm.	5·5
		8	0·0972 „	0·0090 „	8·5
		12	0·0960 „	0·0102 „	9·6
		16	0·0883 „	0·0179 „	16·8
(B) Glycine	15·6 c. c. of N/12 Caustic soda.	4	12·3 c. c.	3·3 c. c.	21·2
		8	11·2 c. c	4·4 c. c.	28·2
		12	10·8 c. c.	4·8 c. c.	30·8
		16	9·95 c. c.	5·65 c. c.	36·3
(C) Potassium oleate.	5·4 c. c. of N/10 $Na_2S_2O_3$.	4	4·5 c. c.	0·9 c. c.	16·7
		8	4·0 c. c.	1·4 c. c.	25·9
		12	3·8 c. c.	1·6 c. c.	29·6
		16	3·2 c. c.	2·2 c. c.	40·7

The above results show that the amount of oxidation increases with the time of exposure.

Comparative Experiments in Sunlight.

TABLE IX

Estimation of (i) glucose, (ii) glycine, and (iii) potassium oleate in concentrated solution in presence of uranium nitrate in sunlight. Air was passed in all the three substances in one set of experiment simultaneously at the same time

of each of the substances taken was practically one gram and the uranium nitrate used was exactly 0·5 grm.

Substance used in the experiment.	Actual amount of the substance taken before the experiment (Blank).	Amount of substance left after the experiment.	Amount of substance oxidised.	Percentage amount of substance oxidised.
1. Glucose ...	0·9620 grm.	0·1342 grm.	0·8278 grm.	85·0
2. Glycine ...	0·9990 grm.	0·2746 grm.	0·7229 grm.	72·5
3. Potassium oleate* ...	56·7 c. c.	16·7 c. c.	40·0 c. c.	70·5

Comparative Experiments with Carbohydrates, Proteins and Fats in Presence of Sunlight.

TABLE X

In this experiment the volume of air passed was 60 litres in 9 hours.

Substance used in the experiment.	Amount of substance taken in 10 c. c. of the solution before experiment (Blank).	Amount of substance left after the oxidation.	Amount of substance oxidised.	Percentage amount of substance oxidised.
1. Butter ...	5·72 c. c. (= 1·10 grm.)	3·9 c. c.	1·82 c. c.	31·8
2. Egg-yellow (5 % solution)	6·9 c. c. (= 0·5 grm.)	2·7 c. c.	4·2 c. c.	60·9
3. Egg-white (5 % solution)	1·6 c. c. (= 0·5 grm.	1·1 c. c.	0·5 c. c.	31·25
4. Starch ...	0·1027 grm.	0·0634 grm.	0·0393 grm.	38·2
5. Glucose ...	0·0962 „	0·0831 „	0·0131 „	13·6
6. Cane sugar	0·0964 „	0·0889 „	0·0075 „	7·8
7. Glycogen ...	0·0987 „	0·0913 „	0·0074 „	7·5

N.B.—The figures in No. 1 represent the amount of absorption of ICl_3 by the butter in terms of N/10 $Na_2S_2O_3$ and in Nos. 2 and 3 the amount of absorption of ammonia by the acid as determined by Kjeldalh's method in terms of N/10 NaOH before and after the experiments.

* The figures indicate the amount of absorption of ICl_3 by potassium oleate in terms of N/10 $Na_2S_2O_3$ before and after the experiments.

The experimental results recorded in Tables VI and VII show that the amount of oxidation determined from the carbon dioxide obtained is practically the same as the oxidation found out from the direct analysis of the carbohydrates, fats and nitrogenous substances remaining unoxidised. Hence in presence of sunlight, different carbohydrates, fats and nitrogenous substances can be completely oxidised by air at the ordinary temperature into their main end products, carbon dioxide and water. No intermediate compounds are formed in these photochemical oxidations. We have thus been able to imitate successfully the physiological processes of oxidation on which animal life depends.

Voit in his necrology of Pettenkofer writes "that the metabolism in the body was not proportional to the combustibility of the substances outside the body, but that protein which burns with difficulty outside metabolises with the greatest ease, then carbohydrates, while fat which readily burns outside is the most difficultly combustible in the body."

We have tried to imitate the metabolism taking place in the animal body and have made comparative experiments on the oxidation of butter, egg-white, egg-yellow, starch, glycogen, glucose and cane sugar by passing air at the ordinary temperature in presence of sunlight. The following results have been obtained:

Egg-yellow	— 60·9%	oxidised.
Egg-white	— 31·25%	,,
Starch	— 38·2%	,,
Butter	— 31·8%	,,
Glucose	— 13·6%	,,

It appears therefore that egg-yellow is the most easily oxidisable substance in presence of light, then comes starch and then egg-white, butter and glucose is the least oxidisable. Hence eggs, which metabolise readily in the animal body, are also easily oxidised by air at the ordinary temperature in presence of sunlight.

Applicability of Einstein Law of Photochemical Equivalence

We have investigated whether the Einstein Law of photochemical equivalence is applicable to the photochemical oxidation of the carbohydrates, fats and nitrogenous substances in sunlight. The amount of energy absorbed by solutions of carbohydrates, fats and nitrogenous substances was estimated with the help of a Boys' radio-micrometer. The experimental results are recorded in the following tables :

(A) *Glycine.*

Amount of glycine oxidised = 0·02325 grm. in $5\frac{1}{2}$ hours in sunlight

(*Vide* Table VII)

Mean wavelength = 5000 Å

Volume taken = 100 c.c.; Area of the surface = 54 sq. cms.

		Deflection	Difference in deflection
Distilled water	(*i*)	34·40	= 34·4 cm.
	(*ii*)	34·40	
			= 0.075 cm.
Glycine	(*i*)	34·30	= 34·325 cm.
	(*ii*)	34·35	

Calculations :—

Energy per quantum corresponding to 5000 Å is $3{\cdot}93 \times 10^{-12}$ ergs per second.

$$u = \frac{C}{\lambda} = \frac{3 \times 10^{10}}{5 \times 10^{-10}} = 6 \times 10^{14}$$

$$\Sigma = hu = 6{\cdot}55 \times 10^{-27} \times 6 \times 10^{14}$$
$$= 3{\cdot}93 \times 10 - {}^{12}$$

Calibration of the scale of the radio-micrometer—Using Hefner lamp :—

Energy per second per square centimeter producing 1 mm. deflection is 1·5 ergs.

(*i*) The number of quanta absorbed by the solute per second per square cm.

$$= \frac{0{\cdot}75 \times 1{\cdot}5 \times 10^{12}}{3{\cdot}93} = 2{\cdot}8 \times 10^{11}$$

(*ii*) Rate of transformation of glycine molecules per second per square cm.

$$= \frac{{\cdot}02325 \times 6{\cdot}06 \times 10^{23}}{330 \times 60 \times 54 \times 1000 \times 75 \times 10}$$

$$= 1{\cdot}7 \times 10^{12}$$

$$\therefore \frac{\text{The number of molecules decomposed}}{\text{The number of quanta absorbed.}}$$

$$= \frac{1{\cdot}7 \times 10^{12}}{2{\cdot}8 \times 10^{11}}$$

$$= 7.$$

(B) *Lactose.*

Amount of lactose oxidised = 0·02 grm. in $5\frac{1}{2}$ hours in sunlight (*vide* Table VII).

Volume taken = 100 c.c.; Area of the surface = 54 sq. cms.

Difference in deflection for lactose = 0·1 cm.

(*i*) The number of quanta absorbed by the solute per second per square cm.

$$= \frac{1 \times 1{\cdot}5 \times 10^{12}}{3{\cdot}93} = 3{\cdot}8 \times 10^{11}$$

(*ii*) Rate of transformation of lactose molecules per second per square cm.

$$= \frac{{\cdot}02 \times 6{\cdot}06 \times 10^{23}}{330 \times 60 \times 54 \times 1000 \times 10 \times 343}$$

$$= 3{\cdot}3 \times 10^{11}$$

$$\therefore \frac{\text{The number of molecules decomposed}}{\text{The number of quanta absorbed}}$$

$$= \frac{3{\cdot}3 \times 10^{11}}{3{\cdot}8 \times 10^{11}} = \mathbf{0{\cdot}87}$$

(C) *Glucose.*

Amount of glucose oxidised = 0·015 grm. in $5\frac{1}{2}$ hours in sunlight (*vide* Table VII).

Volume taken = 100 c.c.; Area of the surface = 54 sq.cms.
Difference in deflection for glucose = 0·1 cm.

(*i*) The number of quanta absorbed by the solute per second per sq. cm.

$$= \frac{1 \times 1{\cdot}5 \times 10^{12}}{3{\cdot}93} = 3{\cdot}8 \times 10^{11}$$

(*ii*) The rate of transformation of glucose molecules per second per sq. cm.

$$= \frac{{\cdot}0151 \times 6{\cdot}06 \times 10^{23}}{330 \times 60 \times 54 \times 1000 \times 180 \times 10} = 4{\cdot}6 \times 10^{11}$$

$$\therefore \frac{\text{The number of molecules decomposed}}{\text{The number of quanta absorbed}}$$

$$= \frac{4{\cdot}6 \times 10^{11}}{3{\cdot}8 \times 10^{11}} = 1{\cdot}21$$

(D) *Alanine.*

Amount of alanine oxidised = 0·0311 grm. in $5\frac{1}{2}$ hours in sunlight (*vide* Table VII).
Volume taken = 100 c.c.; Area of the surface = 54 sq. cm.
Difference in deflection for alanine = 0·2 cm.

(*i*) The number of quanta absorbed by the solute per second per sq. cm.

$$= \frac{2 \times 1{\cdot}5 \times 10^{12}}{3{\cdot}93} = 7{\cdot}6 \times 10^{11}$$

(*ii*) The rate of transformation of alanine molecules per second per square cm.

$$= \frac{{\cdot}0311 \times 6{\cdot}06 \times 10^{23}}{330 \times 60 \times 54 \times 1000 \times 89 \times 10} = 1{\cdot}9 \times 10^{12}$$

$$\therefore \frac{\text{The number of molecules decomposed}}{\text{The number of quanta absorbed}}$$

$$= \frac{1{\cdot}9 \times 10^{12}}{7{\cdot}6 \times 10^{11}} = \mathbf{2{\cdot}5.}$$

It will be interesting to observe that the Einstein Law of Photochemical Equivalence is applicable to the photochemical oxidation of glucose, lactose and alanine by air.

The law, however, is not applicable to the photochemical oxidation of glycine by air where about seven molecules react per quantum of light absorbed. The foregoing results show that practically colourless one per cent aqueous solutions of glucose, lactose, glycine and alanine can absorb light from sunshine falling on the solutions. This absorption of energy leads to the activation of the molecules and their consequent chemical reaction with oxygen in presence of light. When these solutions are mixed with ferric or uranium nitrate, the absorption of radiation is considerably increased and the amount of oxidation is also increased.

Recently we have shown qualitatively that when substances like cholesterol, olive oil are exposed to light in presence of air, peroxides are formed and these can induce the oxidation of food materials mixed with them.

The following quantitative results have now been obtained :

TABLE XI

Estimation of peroxide formed by passing air through mixture of (i) olive oil and water, and (ii) cholesterol and water in presence of sunlight. The volume of air passed was 36·5 litres in $5\frac{1}{2}$ hours. The amounts of olive oil and cholesterol taken were respectively 2·5 grms. and 1 grm. The total volume was made up to 100 c.c.

(1 c.c. of N/10 $Na_2S_2O_3$ = 0·00085 grm. of H_2O_2)

Substance used in the experiment.	Weight of substance taken in the experiment.	Amount of N/10 $Na_2S_2O_3$ required in c.c. before passing air (Blank).	Amount of N/10 $Na_2S_2O_3$ required in c.c. after passing air.	Equivalent amount of H_2O_2 formed in grm. from the compound of the peroxide type.
1. Cholesterol	1·0 grm.	0·0	0·35	0·0002975
2. Olive oil ...	2·5 grm.	0·0	0·50	0·000425
3. Butter ...	0·932 grm.	0·0	0·30	0·000255

These experiments show that an appreciable amount of the substances of the peroxide type is formed when air is passed through the mixture of the above substances in presence of sunlight.

TABLE XII

To estimate the amount of oxidation of glucose by mixing with it the substances of the peroxide type formed by passing air in sunlight through (i) cholesterol and water, and (ii) olive oil and water. The whole system was kept at 40° in a thermostat for 18 hours after which the estimation of glucose was made. The amounts of olive oil and cholesterol taken were 2·5 grms. and 1 grm. respectively.

Substance of the peroxide type used.	Amount of glucose in grm. in 10 c.c. of the solution before experiment.	Amount of glucose in grm. left after the experiment.	Amount of glucose in grm. oxidised.	Percentage amount of glucose oxidised.
1. Olive oil and water.	0·1034	0·1005	0·0029	2·8
2. Cholesterol and water.	0·1034	0·1014	0·0020	1·9

The results show that appreciable amounts of glucose are oxidised by mixing it with the peroxides formed by exposing olive oil and cholesterol to light and air. Hence it is believed that the antirachitic and beneficial properties of substances not containing the necessary vitamins are due to the presence of peroxides, which help the oxidation of food materials in the animal body.

SUMMARY

1. Aqueous solutions or suspensions of the following substances have been oxidised by passing air in presence of sunlight :—sodium formate, sodium tartrate, lecithin, cholesterol, butter, milk, egg white, and egg yellow.

2. Zinc oxide acts as a photo-sensitiser in the oxidation of sodium tartrate and formate and the amount of oxidation is greater than its absence.

3. Photo-chemical oxidations of glucose, starch, glycogen, glycine, α-alanine, hippuric acid, urea, sodium urate, formate, tartrate, potassium stearate and oleate are markedly increased in presence of ferric nitrate and uranium nitrate.

4. Dilute solutions of lactic acid, oxalic acid, tartaric acid and citric acid are appreciably oxidised by air in presence of sunlight and the order in which they are oxidised is :—oxalic > lactic > tartaric > citric.

5. Experimental results on the estimation of carbon dioxide prove that glucose, glycine, potassium oleate, lecithin, cholesterol, butter, etc., are oxidised by air in presence of sunlight chiefly to carbon dioxide and not to any intermediate product.

6. Our experimental results show that the amount of oxidation increases with (i) the amount of light falling on the solutions, and (ii) the time of exposure.

7. Comparative experiments show that the order in which the food materials are oxidised in presence of sunlight is as follows:—Egg-yellow > starch > egg-white, butter > glucose.

8. The Einstein-Law of Photo-chemical Equivalence is approximately applicable to the photo-chemical oxidations of glucose, lactose and alanine by air.

9. Experimental results show that appreciable amounts of the compounds of the peroxide type are formed when air is passed through aqueous suspensions of cholesterol, olive oil and butter. Moreover, appreciable amounts of glucose have been oxidised by mixing the solution of glucose with exposed cholesterol, olive oil and butter containing the peroxide compounds. Hence it is believed that the antirachitic and beneficial properties of substances not containing the necessary vitamins are due to the presence of peroxides which help the oxidation of food materials in the animal body.

COAGULATION OF GELATIN SOLS IN ALCOHOL-WATER MIXTURE

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It is well known that isoelectric gelatin can be precipitated by alcohol. Fenn (Jour. Bio. Chem., 1918, *33*, 279) found that the presence of acids, alkalies and salts hinders the precipitation of gelatin in presence of alcohol. Loeb (Proteins and the Theory of Colloidal Behaviour, 1922, page 252) has similarly shown that gelatin solution of P_H value 4·4 or below, or 5·0 or above can be precipitated with an excess of alcohol, provided the anion of the acid or cation of the alkali added to the isoelectric gelatin is monovalent (*e.g.*, Cl′, CH_3COO', $H_2PO'_4$, $HC_2O'_4$, etc., or Li°, Na°, K°, NH°_4). When, however, these ions are bivalent (*e.g.*, SO''_4, Cä, Bä, etc.), the gelatin can be precipitated with relatively smaller amounts of alcohol. Loeb (ibid., pages 259-260) has also studied the precipitation of both acidic and alkaline gelatin in presence of a large excess of alcohol. The precipitating concentrations of different electrolytes expressed in equivalents with gelatin of P_H value 3·0 in a mixture of alcohol and water are in the following decreasing order :—

$CaCl_3$ > $AlCl_3$ > $LaCl_3$ > $BaCl_2$ > $CaCl_2$ > $SrCl_2$ > $MgCl_2$ > KCl, RbCl, NaCl, HCl, NaBr > LiCl, $NaNO_3$ > HBr, HNO_3, NaI > NaCNS > H_2SO_4 > $Na_2C_2O_4$ > Na_3Citrate > Na_2SO_4, $K_4Fe(CN)_6$

The precipitating concentrations of different electrolytes expressed in equivalents with gelatin of P_H value 10·0 in a mixture of alcohol and water are in the following decreasing order :—

Na_3 Citrate > $Na_4Fe(CN)_6$ > Na_2SO_4 > NAOH > KOH > $Na_2C_2O_4$ > NaCl, NaBr, LiCl > NaCNS >

$NaNO_3$, KCl 7 NaI 7 $Ba(OH)_2$ 7 $Ca(OH)_2$ 7 $CaCl_2$ 7 $SrCl_2$ 7 $CaCl_3$ 7 $BaCl_2$ > $LaCl_3$.

From the above results of Loeb it is to be seen that acid gelatin in alcohol-water mixture behaves like a positively charged sol, whilst the gelatin in the presence of an alkali behaves like a negatively charged sol, and both the colloids obey the Schulze-Hardy Law. It is very interesting to find from the results of Loeb that with acid gelatin far greater amounts of electrolytes of polyvalent cations (*e.g.*, $CaCl_2$, $BaCl_2$, $AlCl_3$, etc.) are required than NaCl, KCl, etc., in spite of the fact that for positively charged sols only chloride ions are potent in coagulating the sols. Polyvalent cations possess an inhibiting action on the precipitation of acidified gelatin in alcohol-water mixture. Similarly, with alkaline gelatin, polyvalent anions from electrolytes like Na_2SO_4, $Na_2C_2O_4$, $Na_4Fe(CN)_6$, etc., also possess an inhibiting action on its precipitation. These results of Loeb seem to indicate that the valency of ions carrying the similar charge as the colloid particles is an important factor in the coagulation of gelatin in alcohol-water mixture.

In previous publications (Jour. Phys. Chem., 1925, *29*, 435, 659 ; 1927, *31*, 187 ; Koll. Zeit., 1924, *34*, 262; 1925, *36*, 131) we have shown that the sols which are capable of adsorbing appreciable amounts of the similarly charged ion from the precipitating electrolyte require larger quantities of the electrolyte for precipitation. We have also proved that these sols show abnormal dilution effect, *viz.*, greater amounts of an electrolyte are necessary for coagulation of diluted sols, than concentrated ones, develop 'ionic antagonism' when coagulated by mixtures of electrolytes and show the phenomenon of positive acclimatization.

In this paper I have, therefore, investigated (*a*) the influence of dilution of the gelatin sol in alcohol-water on its coagulation, (*b*) precipitation with mixtures of electrolytes, and (*c*) the phenomenon of acclimatization.

Positively charged gelatin sol in alcohol-water mixture.—Purified gelatin (Goldruck Kahlbaum) containing 0·1% ash and P_H value 5·3 was used. 5 grams of gelatin were dissolved at 80° in 50 c.c. water and 12·5 c.c. N/8 HCl were added and the total volume was made up to 500 c.c. with absolute alcohol. The P_H value of the same concentration gelatin sol in water in presence of 12·5 c.c. N/8HCl was also determined and found to be 4·40.

The opalescent gelatin sol in alcohol-water mixture was sensitive to electrolytes and it was found that water has a remarkable stabilising influence. 4 c.c. of the gelatin sol were taken in one test tube and 0·5 c.c. of the coagulating electrolytes was taken in another test tube and then mixed up and left for half an hour when the coagulation was observed.

Influenee of dilution on the coagulation of positively charged gelatin sol in alcohol-water mixture.—The sol is diluted with 87·5 % alcohol, which is the amount present in the original sol (Sol A).

TABLE I

Electrolyte.	Amount to coagulate in c.c.		
	Sol A	Sol A/2	Sol A/3
KCl $\frac{N}{10}$	0·26	0·43	7 0·50
K_2SO_4 $\frac{N}{50}$	0·25	0·22	0·18
$K_4Fe(CN)_6$ $\frac{N}{100}$	0·42	0·36	0·30
HCl $\frac{N}{2·5}$	0·35	7 0·50	...
$MgCl_2$ $\frac{N}{2·5}$	0·26	0·48	...

The above table shows that the sol becomes stable on dilution when coagulated by KCl, HCl and $MgCl_2$. The sol could not be coagulated by $Al(NO_3)_3$ and the opalescence disappears when the electrolyte is added to the sol.

Barium chloride also behaves similarly towards the sol which could be coagulated only by a saturated solution of $BaCl_2$. Smaller concentrations of HCl and $MgCl_2$ also make the sol clear.

TABLE II

Coagulation with a mixture of KCl and K_2SO_4.

Amount of N/10	Amount of N/50 K_2SO_4 required for coagulation in c.c.		
N/10 KCl added in c.c.	Observed.	Calculated.	Difference.
0	0·25	...	...
0·26	0	...	...
0·05	0·24	0·20	+0·04
0·10	0·21	0·16	+0·05
0·15	0·17	0·11	+0·06

TABLE III

Coagulation with a mixture of $MgCl_2$ and K_2SO_4.

Amount of N/20 $MgCl_2$ to coagulate in c.c.	Amount of N/50 K_2SO_4 required for coagulation in c.c.		
	Observed.	Calculated.	Difference.
0	0·25	...	...
0·26	0	...	...
0·05	0·26	0·20	+0·06
0·10	0·24	0·16	+0·08
0·15	0·22	0·11	+0·11

TABLE IV

Coagulation with a mixture of $BaCl_2$ and KCl.

$BaCl_2$ N/20 added in c.c.	Amount of KCl N/10 required for coagulation in c.c.
0	0·26
0·05	0·30
0·10	0·38

TABLE V

Coagulation with a mixture of HCl and KCl.

HCl N/2·5 added in c.c.	Amount of N/10 KCl to coagulate in c.c.		
	Observed.	Calculated.	Difference.
0	0·26	...	...
0·35	0	...	...
0·05	0·28	0·22	+0·06
0·10	0·27	0·19	+0·08

TABLE VI

Coagulation with a mixture of HCl and K_2SO_4.

HCl N/2·5 added in c.c.	Amount of N/50 K_2SO_4 to coagulate in c.c.		
	Observed.	Calculated.	Difference.
0	0·25	...	...
0·35	0	...	...
0·05	0·28	0·21	+0·07
0·10	0·28	0·18	+0·10

TABLE VII

Coagulation with a mixture of $Al(NO_3)_3$ and KCl.

$Al(NO_3)_3$ N/20 added in c.c.	Amount of KCl to coagulate in c.c.
0	0·26 of $\frac{N}{10}$
0·05	0·33 of $\frac{N}{10}$
0·10	0·22 of $\frac{N}{5}$

From Tables II, III, IV and VI, it will be seen that ionic antagonism with mixtures of KCl and K_2SO_4, $MgCl_2$ and K_2SO_4, HCl and K_2SO_4, and HCl and KCl. The sol is remarkably stabilised in presence of $BaCl_2$ and Al $(NO_3)_3$ when coagulated by KCl.

In order to investigate the phenomenon of acclimatization by an electrolyte, 4 c.c. of the sol are treated with 0·04 c.c. of the electrolyte at a time after every fifteen minutes to the sol. The coagulation was investigated one hour after the last addition of the electrolyte. The amount necessary to coagulate when added all at once is determined by pouring the whole of the required electrolyte to 4 c.c. of the sol and the coagulation is observed after one hour. The following results have been obtained :—

TABTE VIII

Electrolyte.	Amount required to coagulate when added slowly (0·04 at a time) in c.c.	Amount required to coagulate when added all at once in c.c.
KCl $\frac{N}{20}$	0·36	0·30
HCl $\frac{N}{2·5}$	0·28	0·20
$MgCl_2$ $\frac{N}{5}$	0·38	0·30
K_2SO_4 $\frac{N}{100}$	0·28	0·30

The foregoing tables show that appreciable amount of positive acclimatization is developed when the sol is coagulated by KCl, HCl and $MgCl_2$. Very slight negative acclimatization is developed with K_2SO_4.

Negatively charged gelatin sol in alcohol-water mixture.— ·05 gram was dissolved in 5 c.c. of water at 80° and 2·5 c.c. N/10 KOH. The solution is made up to 500 c.c. with absolute alcohol. P_H value of 0·1% gelatin solution

in presence of 2·5 c.c. N/10 KOH is found to be 7·90. The gelatin sol in alcohol-water mixture is opalescent and the following results were obtained on the coagulation of the sols of different concentrations :—

Influence of dilution on the coagulation of negatively charged sol in alcohol-water mixture.

TABLE IX

The sol is diluted with the same percentage of alcohol-water mixture as present in the original sol :—

Electrolyte.	Amount to coagulate in c.c.		
	Sol A	Sol A/2	Sol A/3
KCl $\frac{N}{10}$	0·32	0·28	0·26
K_2SO_4 $\frac{N}{5}$	0·30	0·32	0·35
$K_4Fe(CN)_6$ $\frac{N}{5}$	0·24	0·25	0·25
$BaCl_2$ $\frac{N}{100}$	0·30	0·18	0·15
$MgCl_2$ $\frac{N}{50}$	0·25	0·18	0·15
KOH $\frac{N}{3·5}$	0·22	0·30	0·36
HCl $\frac{N}{50}$	0·26	0·20	0·15
$Al(NO_3)_3$ $\frac{N}{100}$	0·25	0·20	0·15

The above results show that the sol behaves abnormally towards dilution when coagulated by K_2SO_4, $K_4Fe(CN)_6$ and KOH; on the other hand, it behaves normally towards

dilution when coagulated by KCl, $BaCl_2$, $MgCl_2$ and HCl. When amounts of $Al(NO_3)_3$ or HCl slightly greater than the precipitating concentrations are added to the sol, it becomes clear and charge reversal is observed. Amounts of KOH smaller than the precipitating concentration also make the sol clear.

TABLE X

Coagulation with a mixture of KCl and $BaCl_2$.

Amount of N/10 KCl added in c.c.	Amount of $BaCl_2$ to coagulate in c.c.		
	Observed.	Calculated.	Difference.
0	0·30	...	...
0·32	0	...	...
0·05	0·24	0·25	−0·01
0·10	0·18	0·20	−0·02

There is no 'ionic antagonism' when the sol is coagulated with mixtures of KCl and $BaCl_2$.

TABLE XI

Coagulation with mixtures of K_2SO_4 and $MgCl_2$.

Amount of N/5 K_2SO_4 added in c.c.	Amount of N/50 $MgCl_2$ to coagulate in c.c.		
	Observed.	Calculated.	Difference.
0	0·25	...	...
0.30	0	...	...
0·05	0·21	0·21	0
0·10	0·18	0·17	+0·01
0·15	0·15	0·13	+0·02

Slight 'ionic antagonism' is observed when the sol is coagulated with a mixture of K_2SO_4 and $MgCl_2$.

TABLE XII

Coagulation with mixtures of KOH and KCl.

Amount of N/15 KOH added in c.c.	Amount of N/10 KCl to coagulate in c.c.
0	0·32
0·05	0·35
0·10	0·40

The foregoing results show that the sol is appreciably stabilised towards its coagulation by KCl in presence of KOH.

Experiments on the phenomenon of acclimatization were made with the negatively charged sol and the following results were obtained.

TABLE XIII

Electrolyte.	Amount to coagulate in c.c. when added slowly (0·04 c.c. at a time).	Amount to coagulate in c.c. when added all at once.
KCl $\frac{N}{10}$ ...	0·24	0·25
K_2SO_4 $\frac{N}{10}$ ...	0·28	0·25
$BaCl_2$ $\frac{N}{200}$ ...	0·32	0·40
$MgCl_2$ $\frac{N}{100}$ ...	0·28	0·35
KOH $\frac{N}{3·5}$ ...	0·24	0·15
HCl $\frac{N}{100}$ ...	0·16	0·20
$Al(NO_3)_3$ $\frac{N}{200}$ ...	0·20	0·30

From the above table it will be seen that the phenomenon of positive acclimatization is slightly developed when the sol is coagulated by K_2SO_4 whilst considerable amount

of negative acclimatization is developed when the sol is coagulated by $BaCl_2$, $MgCl_2$, HCl and $Al(NO_3)_3$. The phenomenon of positive acclimatization is, however, developed with KOH.

DISCUSSION

My experimental results with positively charged sols of gelatin in alcohol-water mixture prove that acidified gelatin can appreciably adsorb similarly charged ions from electrolytes like KCl, $BaCl_2$, $MgCl_2$, HCl, $Al(NO_3)_3$, the polyvalent cations and H° ions are adsorbed in larger amounts. Thus the sol could not be coagulated by $BaCl_2$ and $Al(NO_3)_3$ and these electrolytes also exert remarkable antagonistic action towards the coagulation of the sol by KCl. The amounts of HCl and $MgCl_2$ required to coagulate the sol are greater than that of KCl and smaller concentrations of these electrolytes stabilise the sol, and antagonistic action is developed when the sol is coagulated by mixtures of K_2SO_4 and $MgCl_2$, HCl and KCl, and HCl and K_2SO_4. Slight 'ionic antagonism' is also observed when the sol is coagulated with mixtures of KCl and K_2SO_4. The sol becomes stable on dilution when coagulated by KCl, $MgCl_2$ and HCl. It is also observed that KCl, $MgCl_2$ and HCl develops the phenomenon of positive acclimatization with the positively charged sol of gelatin in alcohol-water mixture.

In previous publications I have shown that if an ion carrying the same charge as the colloid particle is appreciably adsorbed by the sol from an electrolyte the following should result :—

(i) Comparatively large adsorption of the similarly charged ions would increase the charge on the colloid particles and consequently inhibit the action of the oppositely charged ion, which causes coagulation;

(ii) The ratio of the amounts of adsorption of the similarly charged ions to that of oppositely charged ions increases

with the dilution of the sol, and this results in the stability of the sol on dilution towards its coagulation by the electrolyte; and

(iii) Greater percentage of adsorption of the similarly charged ions occurs from the smaller concentrations of the electrolyte. This causes 'ionic antagonism,' and develops the phenomenon of positive acclimatization.

Our results, therefore, prove that a sol of acidified gelatin in alcohol-water mixture can adsorb K^0, Ba^{00}, Mg^{00}, H^0 and Al^{000} ions.

From the results of Loeb on the coagulation of negatively charged sol of gelatin in alcohol-water mixture, it will be found that the amounts of K_2SO_4, K_3 citrate, $K_4Fe(CN)_6$, etc., necessary for coagulation are greater than that of KCl. This points to the conclusion that anions SO''_4, $Fe(CN)''''_6$ and $Citrate'''$ are adsorbed in greater amounts than Cl', when the amount adsorbed is expressed in equivalent. Our results on the coagulation of the negatively charged sol with mixtures of KCl and $BaCl_2$ show that no ionic antagonism is developed, and the sol behaves normally towards dilution when coagulated by these electrolytes separately. On the other hand, slight 'ionic antagonism' is developed when the sol is coagulated with mixtures of K_2SO_4 and $MgCl_2$, showing that SO''_4 ions possess an inhibiting action on the coagulation of the sol. The sol also becomes stable on dilution when coagulated by K_2SO_4. Considerable amount of 'ionic antagonism' is developed when the sol is coagulated with mixtures of KOH and KCl. The sol behaves abnormally on dilution when coagulated by KOH. The phenomenon of acclimatization studied with the negatively charged sol of gelatin in alcohol-water mixture shows that whilst positive acclimatization is observed with K_2SO_4 and KOH, slight negative acclimatization is observed with KCl and it is more pronounced with $BaCl_2$, $MgCl_2$, HCl and $Al(NO_3)_3$. In papers published from these laboratories (Jour. Phys. Chem., 1925, *29*, 659; 1927, *31*, 641; Koll.

Zeit., 1926, *37*, 141 ; Jour. I. Chem. Soc., 1928, *5*, 313, we have shown that when a sol can adsorb the oppositely charged ions in large amounts, so that the adsorption of the similarly charged ions is comparatively small, the phenomenon of negative acclimatization is developed. I am, therefore, of opinion that negatively charged sol of gelatin can adsorb appreciable amounts of anions like SO''_4, Citrate$'''$, OH', $Fe(CN)''''_6$ from K_2SO_4, K_3 Citrate, KOH and $K_4Fe(CN)_6$, but the amount of adsorption of anions from KCl, $MgCl_2$, $Al(NO_3)_3$, $BaCl_2$ and HCl is not at all significant in comparison with the amount of adsorption of cations from these electrolytes.

Gelatin, being an amino acid, can behave both as an acid and a base, and, therefore, can adsorb both cations and anions. I have, however, shown in this paper that gelatin prefers to take up a cation more easily than an anion. Thus I have shown in this paper that gelatin sol in alcohol-water mixture can adsorb appreciable amounts of cations like Ba^{00}, Al^{000}, Mg^{00}, etc., whether it is charged positively or negatively, because the acidic character of gelatin is more pronounced than the basic and it exerts an affinity for the adsorption of cations. I have also observed that polyvalent cations are required in very large amounts to coagulate a positively charged sol of gelatin and can very easily bring charge reversal of the negatively charged sols, whilst polyvalent anions cannot bring about charge reversal of the positively charged sol. This is because gelatin can take up more cations than anions. It is well known that the P_H value of the isoelectric gelatin is 4·70 and that gelatin begins to behave as a base, when the medium is distinctly acidic. This also proves that gelatin possesses greater acidic properties than basic and is, therefore, capable of adsorbing comparatively larger amounts of cations than anions.

Fenn (J. Biol. Chem., 1918, *33*, 531) observed the effect of mixtures of salts and acids, and salts and alkalies on the

hindering of precipitation of gelatin by alcohol, and found that salts with bivalent and tervalent cations increase the influence of acid, whilst bivalent and tervalent anions increase the influence of alkali. These results prove that polyvalent cations possess a stabilising influence for the positively charged sol and the polyvalent anions have a stabilising effect on the negatively charged sol of gelatin in alcohol-water mixture. Loeb has shown that isoelectric gelatin is more easily dissolved in an electrolyte containing either polyvalent cation or a polyvalent anion. I am of opinion, that these observations of Fenn and Loeb originate from the property of gelatin, which can adsorb both polyvalent cations and anions.

Perrin (Jur Chim. Physique, *2*, 601, 1904; *3*, 50, 1905) has observed in his experiments on electrical endosmose that salts with trivalent cations reverse the charge of negatively charged membranes and that tetravalent anions cause the reversal of charge of positively charged membranes. Loeb (Jour. Gen. Physiol., 1921-22, *4*, 463) has found that $LaCl_3$ and $CeCl_3$ or $K_4Fe(CN)_6$ makes a film of isoelectric gelatin to assume an electric charge either positive or negative due to the adsorption of La^{000} and Ce^{000} ions or $Fe(CN)''''_6$ ion.

My experimental results on the coagulation of the gelatin sol in presence of either alkali or acid in alcohol-water mixture show that the negatively charged sol can adsorb anions like SO''_4, $Fe(CN)''''_6$, OH', etc., and the positively charged sol can adsorb cations like Ba^{00}, Al^{000}, H^0, etc. Loeb, however, believes that only cations can be taken up by gelatin containing alkali and that only anions can be adsorbed by gelatin containing acid. On the other hand, Pauli (Fotscher. Naturwiss. Forchung, *4*, 223, 1912) holds that both ions of a salt are adsorbed, by a protein. When a block of gelatin is put into a salt solution, it is found that both the ions are present in combination with gelatin. Loeb considers that only one ion can be adsorbed

by gelatin and holds that the presence of the other ion is not due to the actual adsorption by gelatin, but appears because the ion is held within the interstices of the block of gelatin. Loeb took powdered gelatin of different P_H values and treated it with solutions of $AgNO_3$, $NiCl_2$ and $K_4Fe(CN)_6$ separately. The powdered gelatin after being kept in salt solution was washed 8 times with ice-cold distilled water. It was found with isoelectric gelatin at P_H 4·7 that neither the presence of cation nor of the anion could be detected in the gelatin, whilst with gelatin of P_H less than 4·7 only the anion could be tested and with gelatin P_H greater than 4·7 only the cations could be found. Loeb proves from these experiments that only cations could be adsorbed by gelatin of P_H less than 4·7, that only anions could be adsorbed by gelatin of P_H greater than 4·7. Isoelectric gelatin is not able to adsorb a cation or an anion.

I am of opinion that Loeb could not detect the adsorption of cations on the acid side and anions on the alkali side of gelatin because in the course of washing the highly adsorbed water displaced the adsorbed cations and anions. It is well known that substances, which are highly adsorbed by an adsorbent can displace substances which are not highly adsorbed. The adsorption of cations by negatively charged gelatin or of anions by positively charged gelatin cannot be easily displaced by water, because this adsorption occurs due to an affinity existing between colloid particles and the ions and an electric attraction existing between charged gelatin particles and oppositely charged ions. Consequently, it is possible to remove completely the adsorbed similarly charged ion by water because it originates simply from the chemical attraction existing between the colloid particles and the similarly charged ions. The adsorption of oppositely charged ions however cannot be completely displaced by water.

Loeb considers that the effect of ions like Ca^{00}, La^{000}, etc., in inhibiting the precipitation of positively charged gelatin

sol in alcohol-water arises from the increasing effect of these ions on the forces of attraction between water and gelatin. It is generally believed that greater degree of hydration is associated with the stability of the sol. In previous papers (Koll. Zeit., 1928, *44*, 149; Jour. Ind. Chem. Soc., 1928, *5*, 303) we have shown that hydration of sols need not be a significant factor for the stability of sols. Loeb (Proteins and the Theory of Colloidal Behaviour 1922, page 91) has shown that the viscosity of gelatin sol is markedly decreased in presence of neutral salts which shows that the degree of hydration of the sol is decreased by the addition of electrolytes. In several communications (Jour. Phys. Chem., 1925, *29*, 1556; 1926, *30*, 1646; Koll. Zeit., 1928, *44*, 225; 1929) from this laboratory we have shown that sols become less viscous on the addition of small quantities of electrolytes due to the increase of their electric charge by the adsorption of similarly charged ions. Hence the decrease of the viscosity of gelatin by the addition of neutral salts is due to the adsorption of similarly charged ions.

SUMMARY AND CONCLUSION

1. Positively charged gelatin sol in alcohol-water mixture behaves abnormally towards dilution when coagulated by KCl, HCl, and $MgCl_2$, and it could not be coagulated by $BaCl_2$, and $Al(NO_3)_3$.

2. The sol develops 'ionic antagonism' when coagulated by mixtures of KCl and K_2SO_4, $MgCl_2$ and K_2SO_4, HCl and K_2SO_4, and HCl and KCl, and is markedly stabilised in presence of $BaCl_2$ and $Al(NO_3)$ when coagulated by KCl.

3. The above sol shows positive acclimatization when coagulated by KCl, HCl and $MgCl_2$.

4. Negatively charged gelatin sol in alcohol-water mixture behaves abnormally towards dilution when coagulated by KO, K_2SO_4 and K_4 Fe (CN)$_6$ and behaves normally towards dilution when coagulated by KCl.

5. Negatively charged sol shows no 'ionic antagonism' when coagulated by mixtures of KCl and K_2SO_4 and 'ionic antagonism' is observed slightly when coagulated by mixtures of K_2SO_4 and $MgCl_2$. KOH stabilises the sol when it is coagulated by KCl.

6. The phenomenon of positive acclimatization is observed with negatively charged sol when coagulated by K_2SO_4 and KOH, whilst marked negative acclimatization is developed when the sol is coagulated by HCl, $BaCl_2$, $MgCl_2$ and Al $(NO_3)_3$.

7. My results prove that positively charged gelatin can adsorb cations and negatively charged gelatin the anions, but gelatin adsorbs more of a cation than an anion. Similarly charged ions, therefore, play an important role in the coagulation of positively and negatively charged sols of gelatin in alcohol-water mixture.

8. Loeb could not detect the adsorption of cations with gelatin in acid and that of anions with gelatin in alkali because in the course of washing the highly adsorbed water displaced the adsorbed cations and the anions from the positively and negatively gelatin respectively.

VISCOSITY AND SURFACE TENSION OF SATURATED SOLUTIONS OF SALTS

BY

K. P. CHATTERJEE

No work seems to have been done in the determination of viscosity and surface tension of saturated solutions of salts. For this reason, the following investigation is undertaken and the values obtained therein are recorded below.

In the following work, the materials were those of Kahlbaum. They were further recrystallised and the operations were done in silica vessels.

The temperature of the thermostat was maintained constant to ±·03°C. up to about 70°C., varying to only less than ·1°C. near about 90°C.

VISCOSITY

In the thermostat are immersed the solution flask and the viscometer vessel. The solution flask is described later on. Its delivery tube can be connected at will to the intake tube of the viscometer vessel. This is a small vessel through the rubber stopper of which pass a viscometer, a thermometer and another tube opening to the air.

This tube and the one forming the upper part of the viscometer are arranged to have a good length inside the thermostat and then pass out, so that all air going into the viscometer vessel might attain the temperature of the thermostat, and thus the possibility of the accidental fall of temperature is avoided. The above tube of the viscometer rising clear above the thermostat is connected to a suction apparatus and to outside air through a three-way tap. The viscometer is of the kind used by Farrow, but the lower capillary tube is graduated. Through the above

referred three-way tap, the viscometer is connected to an arrangement for suction, different in some respects from that of Farrow. To this, a manometer is attached, so that the suction pressure may be easily brought every time to the same mark.

Before the viscometer is assembled, it is thoroughly cleaned with hot chromic acid and dried by blowing air through it.

Viscosity of H_2O.—The viscosity of water was determined to see what sort of result is obtained and also to calibrate the viscometer tube and to find a constant for it. Conductivity water, distilled in a silica apparatus, was utilised and more than sixteen sets of determinations were done at different times.

One of the graduation marks on the capillary was chosen and the level of the water, or of solution in subsequent operations was always kept at that. The time of rise of water, or of solution in subsequent operations, from this mark to another on the upper tube, under a fixed suction pressure, namely, sixty centimeters of water in manometer, was repeatedly determined and also the time of fall. This was done at different temperatures. The values obtained in one of the sets are given in Table I.

TABLE I

Temperature.	Rise time in seconds.	Fall time in seconds.
20°	126·1	285·8
30°	101·2	229·0
40°	83·3	188·8
50°	70·2	159·4
60°	60·3	137·2
70°	52·8	120·0
80°	46·7	106·7
90°	42·1	95·9

The viscosity was calculated in the usual way by using Scarpa's formula. For water this may be put down as Viscosity at any temperature

$$= \text{Viscosity at, say, } 30°c \times \frac{t_1 \times t_2}{t_1 + t_2} \times \frac{t_3 + t_4}{t_3 \times t_4}$$

where t_1 is the rise time at the temp. of observation;

t_2 is the corresponding fall time,

t_3 is the rise time of water at 30°C,

t_4 is the corresponding fall time, and viscosity of water at 30° is taken as ·008019.

The value of $\cdot 008019 \times \frac{(t_3 + t_4)}{t_3 \times t_4}$ is carefully determined and taking this as the constant of the instrument, the formula may be put down as, Viscosity to be found $= \frac{t_1 \times t_2}{t_1 + t_2} \times C$, where C is the above constant.

As usual with Scarpa's method, the level of water in the viscometer vessel may be kept at any height without much affecting the final result. The values of rise and fall, would of course be quite different, but they come out to be such that in the final result, namely, in the value of the viscosity, very little change is observed. There is some change, undoubtedly, but it is likely to go out if correction figures are introduced.

In subsequent operations with solutions, similar formulæ were used, namely,

$$\frac{\text{Viscosity of sol at any temperature}}{\text{Viscosity of water at that temperature}} = \frac{t_1 t_2}{t_1 + t_2} \times \frac{t_3 + t_4}{t_3 t_4}$$

where t_1 and t_2 are the rise time and fall time of the solution, and t_3 t_4 are the corresponding values for water,
or, viscosity of solution at any temperature

$$= C \times \frac{t_1 t_2}{t_1 + t_2}.$$

By also another formula, the viscosity has been determined.

For instance, in the case of water, it may be put as viscosity of water at any temperature

$$= \text{Viscosity of water at } 30°\text{C} \times \frac{d_1 t_2}{d_2 t_4}$$

where d_1 is the density of water at that temperature,

t_2 is the time of fall of water at that temperature,

d_2 and t_4 are the corresponding quantities at 30°,

and viscosity of water at 30° is taken as ·008019.

Taking $008019 \times \frac{1}{d_2 t_2}$ as a constant, C′, the above becomes

$$\text{Viscosity of water at any temp.} = C' d_1 t_2.$$

This formula involving time of fall alone and density, the latter of course has to be known, and the level of water in the viscometer vessel has always, in all determinations, to be kept at the same mark of the capillary tube. In the case of solution, in subsequent operations, for the same reasons, the density of the solution is to be known and its level has to be kept at the same chosen mark of the capillary tube.

In all readings, by a mechanical arrangement, the parallax is avoided.

The values of viscosity as found by Scarpa's method and also by the other one, are given in Tables II and III.

Viscosity of Saturated Solutions.—A saturated solution of the salt is made in the ususal way at a higher temperature in the solution flask. This is a small pyrex flask with a well-fitting rubber stopper, through which pass a thermometer, a delivery tube and another long one open to the air. This flask is attached to a handle and can be either heated on a flame or kept, with its delivery tube, plugged and immersed in the water of the thermostat. At will, the delivery tube can be connected with the intake tube of the viscometer vessel which has also been kept immersed. When the temperature of the contents of the solution vessel reaches that of the thermostat and of the viscometer, the clear supernatant liquid is blown into the

viscometer vessel until it fills it to the height of the chosen mark on the capillary tube. The whole operation being done under water, the vessel thus can be safely filled with no fluctuation of temperature. The intake tube is then disconnected and plugged.

The rise time and the fall time are carefully found out. The densities are taken from Berkeley's work. In Tables IV and VI are given the times and in Tables V and VII the viscosities as determined by the above referred two ways of calculations.

It must be said, here, that no account has been taken of the kinetic energy factor and of capillary rise (surface tension) and the results are all "uncorrected." Since the timings are large, the first correction is negligible.

TABLE II

Water.

With Log C $= \bar{8}\cdot0580$, and 008019 as viscosity of water at 30°C, the following values were got.

Temperature.	Viscosity of water.	Thorpe and Rodger's values.
20°	·01001	·01005
30°	·008019	·008019
40°	·006606	·006588
50°	·005569	·005537
60°	·004786	·004752
70°	·004191	·004144
80°	·003713	·003655
90°	·003344	·00326

TABLE III

At the chosen level 16, the value of log C′ was got as $\bar{8}$·5462. With it, the following values of viscosity of water were obtained:—

20°	·01003
30°	·008019
40°	·006591
50°	·005540
60°	·004744
70°	·004128
80°	·003648
90°	·003256

For other levels, the constants would be different.

TABLE IV

A saturated solution of sodium chloride.

Temperature.	Rise time.	Fall time.	Density of the solution.
30°	223·8	384·2	1·1956
50°	183	316·1	1·1912
60°	153·8	266·8	1·1868
70°	132·2	231·1	1·1826
80°	115·0	202·0	1·1784
80°	102·0	179·0	1·1744
90°	91·2	161·3	1·1704

TABLE V

Viscosity of saturated solution of sodium chloride.

Log C = $\bar{8}$·0580 as used in A.

Log C′ = $\bar{8}$·5462 as used in B.

Temperature.	A.	B.
30°	016150	016150
40°	013250	013240
50°	011140	011140
60°	009610	009612
70°	008375	008371
80°	007427	007398
90°	006664	006667

TABLE VI

Saturated potassium chloride solution.

Temperature.	Rise time.	Fall time.	Density.
30°	125·1	218·7	1·182
40°	109·8	190 4	1·1877
50°	98·5	169·7	1·1931
60°	89·6	153·3	1·1982
70°	82·4	139·4	1·2018
80°	76·2	128·9	1·2046

TABLE VII

Viscosity of saturated potassium chloride solution.
Log C $= \bar{8}\cdot0580$, as used in A.
Log C^1 $= \bar{8}\cdot5462$, as used in B.

Temperature.	A.	B.
30°	09095	09095
40°	07962	07958
50°	07123	07120
60°	06463	06460
70°	05886	05884
80°	05474	05462

Other solutions are being investigated and a more refined instrument is used.

SURFACE TENSION OF SATURATED SALT SOLUTIONS

The same solution flask is used as was done in the viscosity experiment. Its delivery tube is arranged so

that it can get connected to the intake tube of the surface tension vessel, when these are immersed in the thermostat. This surface tension vessel is a vessel of small size, through the rubber stopper of which pass a thermometer, a long tube open to the air and another one through which a finely graduated capillary tube can be slid in and held at any position by a small rubber sleeve. This graduated capillary tube is thoroughly cleaned with chromic acid mixture and dried, before it is put in.

The solution is made in the usual way in the solution flask and then it is brought and kept immersed in the thermostat. When the temperature of the contents of the solution flask reaches that of the thermostat and of the surface tension vessel, the clear supernatant solution is blown into the latter and the whole operation being done inside the thermostat no fluctuation of temperature is brought about. The intake tube is then disconnected and plugged. The capillary tube is then slid in until its end dips about 5 mm. below the surface of the liquid in the surface tension vessel. Some air is now blown in through the tube which was stated as open to the air. This tube being a long one and tortuous in its path, the air gets heated to the temperature of the thermostat by the time it comes in contact with the liquid in the surface tension vessel, and thus no fluctuation of temperature is produced. The air pressure thus created will make the liquid rise up quite high in the capillary tube. The pressure is now released, and the head of the liquid falls down to some point in the capillary. From the graduations, the height of the liquid is thus found out.

From the formula, namely, surface tension $= \frac{1}{2}$ grdh, where

g is acceleration due to gravity,

r is the radius of the bore,

d is the density of the liquid,
h is height of the liquid

and from the accepted value of surface tension, at 30°, of water, the value of $\frac{1}{2}gr$ is deduced. Finding this as 0·4239, the formula for surface tension may be put down as

Surface tension = 0·4239 × height × density.

In the following tables no account is taken of the increase of r and h due to increase of temperature and the values are therefore uncorrected.

TABLE I

Surface tension of water.

Temperature.	Height.	Density.	Calculated surface tension.	Values in reference books.
20·6	171·2	·9982	72·43	...
30	168·3	·9957	71·03	71·03
40	165·2	·0022	69·49	69·54
50	162	·9881	67·85	67·80
60	158·6	·9832	66·13	66·00
70	155·1	·9778	64·30	64·20
80	151·5	·9718	62·41	62·30

TABLE II

Surface tension of a saturated solution of potassium chloride.

Temperature.	Height.	Density.	Calculated surface tension.
20·6	156·6	1·1746	77·94
30	154	1·182	77·18
40	152	1·188	76·56
50	150	1·1931	75·91
60	147·7	1·1982	75·02
70	145·5	1·2020	74·15
81·5	143·2	1·205	73·13
83	142·5	1·2053	72·78

TABLE III

Surface tension of a saturated solution of sodium chloride.

Temperature.	Height.	Density.	Calculated surface tension.
30°	159·5	1·1956	80·85
40°	157	1·1912	79·27
50°	155	1·1868	78·00
60°	153	1·1826	76·72
70°	151	1·1784	75·44
80°	149	1·1744	74·18
90°	147	1·1704	72·93

Other solutions are being investigated.

DIALYSIS, ULTRA FILTRATION AND CO-AGULATION WITH MOLYBDIC ACID

BY

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In previous publications[1] we have observed that the sols of vanadic, tungstic and antimonic acids show an increase in viscosity, decrease in the electric conductivity and become unstable toward their coagulation by electrolytes on ageing. We have shown that these acids when freshly prepared exist partly in the molecular condition. In course of time the particles in the molecular condition polymerise to form colloids. We have found with a dialysed sol of vanadic acid that it leaves 5·1 % of vanadic acid in solution after the colloid is completely coagulated by KNO_3 and when the dialysed sol of vanadic acid is kept for 32 days before coagulation by KNO_3 the amount of vanadic acid in the dissolved condition becomes 4·4 %. Similarly we have observed with antimonic acid sol that more than 50% of antimonic acid is in solution when first prepared by dropping $SbCl_5$ in distilled water, and this amount rapidly passes to the colloidal state practically completely when kept for a week (also compare Delacroix Bull. Soc. Chim., *21* (iii) (1899), 1049, *25* (1901), 283· and Senderens).[2] Mylius and Groschuff[3] working with silicic acid sol observed a similar behaviour. Similarly Gutbier and Brintzinger[4] showed that silicic acid is capable of diffusion when freshly prepared and cannot diffuse after some time as the particles grow. Evidence of the growth of the particles on ageing has also

[1] Jour. Phys. Chem., 1929; Jour. Ind. Chem. Soc., *6* (1929), 17.
[2] Ibid., *21* (iii) (1899), 47.
[3] Berlin, 39,111 (1906), 116.
[4] Z. anorg. and Alleg. Chem., *159* (1927), 231.

been obtained by us with silicic, tungstic, vanadic and antimonic acids.

In this paper we have shown that molybdic acid obtained by the interaction of ammonium molybdate and an equivalent amount of hydrochloric acid contains a large proportion of molybdic acid in molecular condition, which gradually polymerises to the colloidal state. The following experiments were performed on the diffusion of molybdic acid through a parchment bag :

To 150 c.c. of a solution containing 3 grams of ammonium molybdate, 150 c.c. of N/9·75 HCl were added. Molybdic acid is obtained according to the equation

$$(NH_4)_6\ Mo_7\ O_{24} + 6HCl + 4H_2O = 6\ NH_4Cl + 7H_2MO_4$$

As the amount of HCl added is equivalent to that of ammonium molybdate, the mixture thus prepared contains NH_4Cl and molybdic acid, and is optically clear and is acidic to litmus. The P_H value as determined by a quinhydrone electrode was 1·873. 100 c.c. of this solution was kept for dialysis in a parchment bag surrounded by 200 c.c. of distilled water in a beaker at 30°. At the outset of the experiment the levels of water within the parchment bag and that in the beaker were the same, but after 10 hours of dialysis it was found that some water has rushed into the bag because of osmosis. It was also observed that after 10 hours of dialysis the diffusion of molybdic acid has reached a maximum as will be evident from the following table :

TABLE I

Time.	Amount of molybdic acid inside the parchment bag per 50 c.c. of the solution.
10 hours	0·294 grm. MoO_3
15 hours	0·294 grm. MoO_3

Measurable amounts of molybdic acid came out in the dialysate during dialysis and after 10 hours of dialysis

both the inside and outside solution were estimated and their contents of MoO_3 determined. A measured volume of this dialysed solution was redialysed for 10 hours being surrounded with twice its volume of distilled water. The MoO_3 content in the dialysed and dialysate solutions is again estimated. The experiment was repeated till molybdic acid was found to be free from chloride. The results are given below:

TABLE II

Amount of MoO_3 present in the original mixture = 0·815 grm. MoO_3 per 10 c.c.

Number of dialysis.	Amount of MoO_3 in grms. per 50 c. c. of the solution inside the bag.	Amount of MoO_3 in grms. per 50 c. c. of the solution outside the bag.	Ratio of the amount of MoO_3 in the dialysed sol to that in the dialysate.
1	0·282	0·0340	8·3
2	0·236	0·0244	9·6
3	0·218	0·0208	10·5
4	0·174	0·0142	12·3
5	0·152	0·0114	13·7

The following results were obtained when molybdic acid obtained by the reaction of ammonium molybdate and HCl was kept for 10 days before dialysis.

TABLE III

Number of dialysis.	Amount of MoO_3 in grms. per 50 c. c. of the solution inside the bag.	Amount of MoO_3 in grms. per 50 c. c. of the solution outside the bag.	Ratio of the amount of MoO_3 in the dialysed sol to that in the dialysate.
1	0·320	0·0304	10·5
2	0·256	0·0230	11·1
3	0·226	0·0176	12·8
4	0·200	0·0130	15·4
5	0·170	0·0094	18·1

The same sol when kept for 20 days before dialysis gave the following results for the first dialysis.

TABLE IV

Number of dialysis.	Amount of MoO_3 in grms. per 50 c. c. of the solution inside the bag.	Amount of MoO_3 in grms. per 50 c. c. of the solution outside the bag.	Ratio of the amount of MoO_3 in the dialysed sol to that in the dialysate.
1	0·328	0·0240	13·7

These results recorded in the above tables indicate that appreciable amounts of molybdic acid are present as true solution which can diffuse through a parchment bag and as the process of dialysis is continued the amounts of molybdic acid which can diffuse decrease as the content of molybdic acid present as colloid increases. It will be also apparent from the above tables that the diffusion of molybdic acid is always less in the case of an aged sol than a fresh one. Thus the amount of molybdic acid which can diffuse through the parchment bag is 1/8·3 parts of the molybdic acid present inside the bag after 10 hours of dialysis when it is freshly prepared, whilst it is 1/10·5 parts after 10 days of its preparation and 1/13·7 after 20 days of its preparation.

We have also investigated the influence of an excess of an acid and an alkali on the diffusion of freshly prepared molybdic acid and the following results were obtained on the dialysis of the solution :

To 150 c.c. of molybdic acid solution prepared by the interaction of 75 c.c. of 2% ammonium molybdate and 75 c.c. of equivalent amount of hydrochloric acid, 4·7 c.c. NHCl were added.

TABLE V

Number of dialysis.	Amount of MoO_3 in grms. per 50 c. c. of the solution inside the bag.	Amount of MoO_3 in grms. per 50 c. c. of the solution outside the bag.	Ratio of the amount of MoO_3 in the dialysed sol to that in the dialysate.
1	0·310	·0310	10·00
2	0·266	·0210	12·66

To 150 c.c. of molybdic acid prepared as given above, 4·7 c.c. N NH_4OH were added.

TABLE VI

Nmber of dialysis.	Amount of MoO_3 in grms. per 50 c. c. of the solution inside the bag.	Amount of MoO_3 in grms. per 50 c. c. of the solution outside the bag	Ratio of the amount of MoO_3 in the dialysed sol to that in the dialysate.
1	0·270	0·350	7·7
2	0·220	·0252	8·8

The foregoing results show that more of molybdic acid diffuses in the presence of an alkali than in the presence of an acid.

In order to show that considerable amounts of molybdic acid are present in the molecular condition, some experiments were made on ultra filtration. A small filter paper fitting a conical Buchner funnel was washed with hot water, and the moist filter paper was dipped in 4% collodion solution in ether-alcohol mixture and the excess of the collodion solution is drained off. On evaporation of ether and alcohol a fine coating of collodion was obtained. This process was repeated three times and the filter paper thus coated with

collodion was kept on the perforated bottom of the funnel. The sides of the filter paper were made air-tight by means of collodion solution. The ultra filtration was quickened by a water pump. The ultra filter thus prepared could check colloidal particles of ferric hydroxide sol prepared from ferric chloride solution added to boiling water.

To 500 c.c. of the solution containing 10 grams of ammonium molybdate 30·9 c.c., 1·5715 NHCl were added, so that the acid added was in equivalent amount. About 50 c.c. of the molybdic acid thus produced were taken after 2 hours for ultra filtration and it was found that considerable amount of molybdic acid appeared in the filtrate, which was estimated as MoO_3. At the beginning of the experiment two filter papers were coated with collodion as already described and the amounts of molybdic acid in the filtrate from two different ultra filters were estimated and the results are recorded below :

TABLE VII

Ultra filter number.	Amount of MoO_3 in grams. per 100 c.c. of the filtrate.
1	0·740
2	0·740

The above results, therefore, prove that the pores of the different ultra-filters as prepared by the method already described are practically of the same magnitude. The mixture of molybdic acid and ammonium chloride was kept and the amounts of molybdic acid in the aged samples were estimated from day to day after ultra filtration. The following results were obtained :

TABLE VIII

Amount of MoO_3 in 100 c.c. of the mixture = 1·550 grms.

Time in hours.	Amount of MoO_3 in grms. present per 100 c.c. of the filtrate.	Amount of MoO_3 in grms present as per colloid 100 c. c. of the mixture.	Percentage of MoO_3 as colloid.
2	0·740	0·810	52·2
24	0·732	0·818	52·77
48	0·726	0·824	53·16
72	0·714	0·832	53·67
96	0·706	0·840	54·19
120	0·700	0·846	54·59

The influence of an acid and a base on the polymerisation of molybdic acid sol was also investigated and the following results were obtained :

TABLE IX

5 c.c. of 1·5715 NHCl were added to 300 c.c. of acid molybdic as prepared in the previous experiment.

Amount of MoO_3 in 100 c.c. of the mixture = 1·528 grms.

Time in hours.	Amount of MoO_3 in grms. per 100 c c. of the filtrate.	Amount of MoO_3 in grms. present as colloid per 100 c. c. of the mixture.	Percentage of MoO_3 present as colloid.
2	0·736	0·792	51·83
24	0·720	0·808	25·94
48	0·703	0·825	53·99
72	0·690	0·838	54·91

TABLE X

5 c.c. of 1·57 N NH_4 OH were added to 300 c.c. of molybdic acid.

Amount of MoO_3 in 100 c. c. of the mixture = 1·528 grms.

Time in hours.	Amount of MoO_3 in grms. per 100 c. c. of the filtrate.	Amount of MoO_3 as colloid in grms. per 100 c. c. of the mixture.	Percentage of MoO_3 present as colloid
4	0·766	0·762	49·86
24	0 760	0·768	50·25
48	0·752	0·776	50·78
72	0·746	0·782	51·18

The results recorded in Tables VIII, IX and X show that the amount of colloidal MoO_3, which can be checked by ultra-filtration increases with time. It will be also seen that the presence of an acid increases aggregation to a greater extent than the presence of an alkali.

It will be interesting to note here that Schulz and Jander[1] from diffusion experiments with sodium tungstate and Dumanski and co-workers[2] with sodium tungstate and molybdate have observed increased polymerisation of tungstic and molybdic acids on the addition of acids.

The amounts of colloidal aggregates of molybdic acid in solutions of different concentrations have been also investi gated and the following results were obtained :

TABLE XI

Equivalent amounts of HCl were added to ammonium molybdate.

	Amount of MoO_3 in grms. per 100 c. c. of the solution.	Amount of MoO_3 in grms. present per 100 c. c. of the filtrate.	Amount of MoO_3 in grms. present as colloid per 100 c. c. of the solution.	Percentage of colloid.
2% sol.	1·55 grms.	0·740	0·810	52·2
1% sol.	0·775 grm.	0·508	0·267	34·4
½% sol.	0·3875 grm.	0·296	0·0915	23·9

[1] Z. anorg. und Alleg. Chem., *162* (1927), 141.
[2] Jour. Russ. Phys. Chem., *58*, 107 (1926), 326.

The above table proves that the bigger aggregates of molybdic acid become smaller as the solution is diluted.

We have also investigated the influence of ageing on the change in the specific conductivity of a solution of molybdic acid and ammonium chloride obtained by the interaction of equivalent amounts of ammonium molybdate and hydrochloric acid. The results are given below:—

TABLE XII

150 c.c. of solution containing 3 grams of ammonium molybdate and 150 c.c. N/9·75 HCl.

Date.	Specific conductivity $\times 10^{-3}$ at 30°.
17-12-28	13·29
19-12-28	13·01
21-12-28	12·89
23-12-28	12·67
25-12-28	12·36
27-12-28	11 85

The following results were obtained regarding ageing on the change in specific conductivity of a solution of molybdic acid dialysed free from NH_4Cl.

TABLE XIII

Concentration of the solution 3·04 grms. MoO_3 per litre.

Date.	Specific conductivity $\times 10^{-3}$ at 30°.
27-12-28	1·719
31-12-28	1·676
4-1-29	1·660

The following measurements of electric conductivity are with the dialysate obtained after 10 hours of dialysis of a

mixture of molybdic acid and ammonium chloride. This dialysate contains chiefly ammonium chloride and molybdic acid in true solution.

TABLE XIV

Amount of MoO_3 in 100 c.c. of the dialysate = 0·068 grm.

Date.	Specific conductivity $\times 10^{-3}$ at 30°.
19-12-28	4·38
21-12-28	4·34
23-12-28	4·25
25-12-28	4·19
27-12-28	4·04

Tables XII, XIII and XIV show that there is a marked decrease in the specific conductivity of molybdic acid solutions on keeping. We are of opinion that this decrease which is similar to that observed in the case of sols of vanadic, tungstic, and antimonic acids originates from the tendency of molybdic acid, which is present in the molecular condition to polymerise and form bigger aggregates on ageing.

We have shown that sols of silicic, vanadic and tungstic acids are sensitized towards their coagulation by electrolytes in presence of a trace of an alkali and we have pointed out that this happens because a part of these acids exists in the molecular condition. The stability of these colloids is due to the adsorption of the complex anion of the acids in solution. On the addition of a few drops of alkali, the complexity of these anions decreases and hence the amount of adsorption and the stability decreases.

In the foregoing pages we have shown that a large percentage of molybdic acid is present in molecular condition, and this has a tendency to polymerise. We have, now,

made some coagulation experiments with a freshly prepared molybdic acid sol in presence of traces of ammonia. P_H values were also determined with a quin-hydrone electrode. For coagulation only monovalent cations were used as the bivalent cations form insoluble molybdates. The results are as follows:

TABLE XV

Concentration of the sol = 15·50 grms. of MoO_3 per litre.
Amount of solution taken each time = 3 c.c.
Total volume = 5 c.c.; Time of observation = 30 minutes.

N/2NH_4OH added in c.c.	Electrolytes necessary for coagulation.					P_H value
	4·65 N LiCl	3N NaCl	3N KCl	N NH_4Cl	N/6 RbCl	
0·0 c.c.	0·6 grm. solid.	1·7 c.c.	0·55 c.c.	1·0 c.c.	0·7 c.c.	1·873
0·04	0·55 grm. solid.	1·65 c.c.	0·50 c.c.	·9 c.c.	0·65 c.c.	2·012
0·08	0·5 grm.	1·60 c.c.	0·45 c.c.	·8 c.c.	0·6 c.c.	2·169
0·10	0·48 grm.	1·55 c.c.	0·40 c.c.	·7 c.c.	0·55 c.c.	2·237
0·12	0·45 grm.	1·50 c.c.	0·35 c.c.	·65 c.c.	0·50 c.c.	2·324
0·16	2 c.c.	1·45 c.c.	0·30 c.c.	·60 c.c.	0·45 c.c.	2·532
0·20	0·5 grm. solid.	2·00 c.c.	0·80 c.c.	·90 c.c.	0·80 c.c.	2·740
0·24	0·7 grm. solid.	2·00 c.c.	1·8 c.c.	1·30 c.c.	1·8 c.c.	3·082
0·28	>0·7 grm.	>2·00 c.c.	1·0 grm. solid.	1·9 c.c.	>2 c.c.	3·608
0·32	>0·7 grm.	>2·00 c.c.	>1·5 grms. solid.	1·3 c.c. of 2N NH_4Cl	>2 c.c.	4·039
0·36	>1·0 grm.	>1·0 grm. solid.	>2 grms. solid.	>2 c.c. 2N NH_4Cl	>2 c.c.	4·28

A perusal of the above table will show that the coagulation of the molybdic acid is remarkably sensitized

by the first few drops of NH_4OH, whilst larger amounts hinder the coagulation.

The coagulation power of the monovalent cations is in the following decreasing order :—

$$Rb^{\cdot} > NH^{\cdot}_4 > K^{\cdot} > Na^{\cdot} > Li^{\cdot}.$$

This order of coagulating powers of monovalent cations is also found with several negatively charged sols.

We have found that when molybdic acid is diluted it becomes very stable and could not be easily coagulated by electrolytes as will be shown from the following table :

TABLE XVI

Original solution containing 15·50 grms. of MoO_3 per litre is diluted three times.

Electrolytes.	Amount necessary to coagulate.
LiCl	Saturated solution.
NaCl	Saturated solution.
KCl	2 c.c. of saturated solution.
NH_4Cl 4N	1·5 c.c.
RbCl $\frac{N}{6}$	1·5 c.c.

The table shows that the sol becomes very stable on dilution. In previous publications, we have shown that the stability of a sol on dilution towards its coagulation by an electrolyte originates from the adsorption of similarly charged ions. We have, however, found that a sol of benzopurpurin is not capable of adsorbing the similarly charged ions appreciably but becomes stable on dilution. We have shown that this happens because the colloidal particles become smaller in size or dilution. In this paper

we have shown that the percentage of colloidal molybdic acid, that can be checked by ultra filtration, remarkably decreases in the case of diluted solutions of molybdic acid. We are of opinion that molybdic acid exists more in colloidal condition in a concentrated solution and this becomes smaller and approaches molecular size as the solution is diluted. In a recent paper, Furth and Ullman[1] have shown that the size of colloidal particles from neutral red and trypan red decreases with dilution, and at infinite dilution the radius of the colloidal particles approaches the molecular size.

In this connection it is interesting to note that Biltz and Vegesock[2] and Bayliss[3] showed that the ratio of the osmotic pressure and the concentration increases in the case of dialysed congo-red sol on dilution. Similar results have been obtained by Moore and Parker[4] with sodium oleate and Reid[5] with crystallised hemoglobin. These results can be satisfactorily explained from the viewpoint that on dilution these substances pass from the colloidal to the molecular condition. On the other hand, the ratio of the osmotic pressure to the concentration with sols of ferric hydroxide, thorium hydroxide, etc., decreases on diluting the sols (compare Duclan[6]) and we have shown in these laboratories that these sols are not stabilised on dilution. Hence, there is considerable evidence in favour of the view that benzopurpurin, congo red, molybdic acid and other allied substances disintegrate on dilution. Our results obtained in this paper support the conclusions arrived at previous one with benzopurin sols and are not in agreement with the views of Weigner and Marshall.[7]

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[1] Koll. Zeit., *41* (1927), 304.
[2] Zeit fur. Phys. Chem., *68* (1909), 357; 73 (1910), 481.
[3] Proc. Roy. Soc., *81* (1909), 269; Koll. Zeit., *6* (1910), 23.
[4] Amer. Journal Physiol., I (1902), 261.
[5] Journ. Physiol., *2* (1906), 34.
[6] Koll. Zeit., *3* (1908), 126.
[7] Zeit. fur Phys. Chem., *140* (1929), 55.

SUMMARY

1. The following conclusions can be drawn from our experiments on the dialysis and ultrafiltration of a solution of molybdic acid obtained by the interaction of ammonium molybdate and hydrochloric acid :

(*a*) Molybdic acid solution when freshly prepared contains considerable amounts of the substance in the molecular condition.

1·550 grms. of molybdic acid in 100 c.c. of solution contain as much as 47·8 % of the substance in the molecular condition, which could not be checked by an ultra filter.

(*b*) Amount of molybdic acid present in the molecular condition gradually decreases with time.

(*c*) Addition of an acid to molybdic acid solution increases the colloid content whilst the addition of an alkali decreases it.

2. The specific conductivity of molybdic acid decreases on ageing because the acid existing in the molecular condition gradually polymerises with time.

3. Coagulation of molybdic acid sol has been effected with monovalent cations and their coagulating powers are in the following decreasing order :

$$Rb^{\cdot} > NH_4^{\cdot} > K^{\cdot} > Na^{\cdot} > Li^{\cdot}.$$

4. Traces of an alkali like NH_4OH sensitizes the sol towards its coagulation by monovalent cations, whilst larger amounts of NH_4OH stabilise the sol. We are of opinion that this is due to the fact that an acid increases and an alkali decreases the polymerisation of the complex molybdic acid present in the molecular condition.

5. Molybdic acid solution when diluted becomes very stable towards its coagulation by electrolytes. We are of opinion that colloidal particles of molybdic acid disintegrate and finally approach molecular size on dilution.

6. It has been shown by ultra filtration that the percentage of colloid content in a molybdic acid solution decreases on dilution.

7. We are of opinion that the increases in ratio of the osmotic pressure to the concentration as observed with congo red, sodium oleate, hemoglobin, etc., on dilution are due to the disintegration of the colloid particles on dilution.

INFLUENCE OF LIGHT ON COLLOIDS

BY

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In publications from these laboratories the influence of light on some properties of colloids has been investigated. We have observed that sols can be divided into two classes. In one class the stability of the sol towards electrolytes increases and in the other the stability decreases on exposure to light. With some sols the electric conductivity increases whilst with others it decreases on exposure to light. Measurements of the absorption spectra of the exposed and unexposed sols showed more marked absorption of light in the cases of ferric hydroxide, arsenious sulphide and prussian blue and less absorption in the cases of gum dammar, mastic, etc. We have also shown that sols of manganese dioxide, ceric hydroxide, copper ferrocyanide and oden sulphur coagulate completely on exposure to sunlight.

In this communication we have investigated the influence of sunlight and light from a thousand watt gas filled tungsten filament lamp on (*a*) the coagulation, (*b*) electric conductivity, and (*c*) extinction coefficient of sols of stannic hydroxide, aluminium hydroxide, thorium hydroxide, antimony sulphide, mercuric sulphide, arsenious sulphide, cupric ferrocyanide, uranium-ferrocyanide.

The sols were exposed to light in small stoppered Jena glass bottles or silica vessels. The extinction coefficients were determined by a Nuttings Spectro-photometer.

STANNIC HYDROXIDE

The stannic hydroxide was prepared at the ordinary temperature by adding ammonium hydroxide to stannic

chloride. The stannic hydroxide thus obtained was washed two or three times to free it from electrolytes partially. On further addition of water it turned immediately to a sol. The sol was dialysed for ten days.

TABLE I

Concentration =6·66 grms. of SnO_2 per litre
Volume of mixture =10 c.c.
Amount of sol taken =1 c.c. of stannic hydroxide
Time allowed for coagulation =1 hour

Electrolyte used.	Amount in c.cs needed for coagulation.	
	Unexposed.	Exposed Sol. was exposed for 169 hours and 15 minutes.
KCl N/2·5 ...	0·80	0·55
$BaCl_2$ N/500 ...	2·50	2·00

ELECTRIC CONDUCTIVITY AT 25°C.

Sp. conductivity for unexposed sol.	Sp. conductivity for sol exposed for 237 hours.
0·005794 mhos.	0·006164

EXTINCTION COEFFICIENTS.

Sol taken.	Extinction coefficient.
Unexposed SnO_2 ...	0·0
SnO_2 exposed to sunlight for 201 hours and 40 minutes ...	0·1

ALUMINIUM HYDROXIDE

Aluminium hydroxide was precipitated by the reaction of ammonium hydroxide on aluminium nitrate solution at the ordinary temperature. The precipitate was completely freed from electrolytes by repeated washing and was peptised to a turbid sol by the addition of a few drops of dilute hydrochloric acid.

TABLE II

Concentration = 15·42 grms. of Al_2O_3 per litre
Total volume taken = 10 c.c.
Amount of sol taken = 0·5 c.c. of $Al(OH)_3$
Time allowed for coagulation = 1 hour

Electrolyte used.	Amount of electrolyte required for coagulation given in c.c.	
	Unexposed sol.	Sol exposed for 85 hours.
KCl N ...	3·25	3·0
K_2SO_4 N/500 ...	4·10	4·0

ELECTRIC CONDUCTIVITY AT 25°

Sp. conductivity for sol kept in the dark.	Sp. conductivity for sol exposed to sunlight for 133 hours and 25 minutes.
0·001821	0·001888

EXTINCTION COEFFICIENT

Sol.	Extinction coefficient.
Unexposed $Al(OH)_3$ ...	0·8
$Al(OH)_3$ exposed for 94 hours	0·72

THORIUM HYDROXIDE

A solution of Kahlbaum's pure thorium nitrate was filtered and boiled for about an hour, and the boiled solution was subjected to hot dialysis for seven days. A clear transparent sol of thorium hydroxide was obtained.

TABLE III

Concentration =6·1 grms. of ThO_2 per litre
Volume of mixture =10 c.c.
Amount of sol taken =0·5 c.c.
Time allowed for coagulation =1 hour

Electrolyte used.	Amount in c.c needed for coagulation.	
	Unexposed sol.	Sol exposed for 58 hours and 45 minutes.
KCl 5·33 N ...	1·85 c.c.	1·25 c.c.
K_2SO_4 N/50 ...	0·60 c.c.	0·50 c.c.
Potassium citrate N/100 ...	1·30 c.c.	0·60 c.c.

ELECTRIC CONDUCTIVITY.

Sp. conductivity for sol kept in the dark.	Sp. conductivity for sol exposed for 106 hours and 10 minutes.
0·009581	0·01284
0·009796	0·01278

EXTINCTION COEFFICIENTS.

Sol of $Th(OH)_4$	Extinction coefficients.
Sol kept in the dark ...	0·03
Sol exposed for 75 hours and 30 minutes	0·07

MERCURIC SULPHIDE

Mercuric oxide was precipitated by the reaction of a solution of pure potassium hydroxide on a solution of mercuric chloride. The precipitate was freed from alkali and chloride by repeated washing, first with cold and then with hot water. The purified mercuric oxide was boiled in a Jena flask with conductivity water. To the saturated solution of mercuric oxide thus obtained a slow current of hydrogen sulphide was passed and a black sol of mercuric sulphide was obtained. The excess of hydrogen sulphide was removed by passing a current of hydrogen. By this method one can readily obtain a pure sol of mercuric sulphide.

TABLE IV

Concentration of sol. = 0·11 grms. of HgS per litre
Volume of mixture = 5 c.c.
Amount of sol. taken = 2·5 c.c.
Time allowed for coagulation = 1 hour

Electrolyte used.	Amount in c.c. needed for coagulation.	
	Unexposed sol.	Sol exposed for 88 hours 15 minutes.
KCl N/10	1·2 c.c.	0·4 c.c.
$BaCl_2$ N/40	0·3 c.c.	0·15 c.c.

ELECTRIC CONDUCTIVITY.

Sp. conductivity for sol kept in the dark.	Sp. conductivity for sol exposed for 147 hrs. 45 minutes.
0·0002146	0·0002869

EXTINCTION COEFFICIENTS.

Sol taken.	Extinction coefficients.
HgS kept in the dark ...	1·25
HgS exposed for 117 hours and 5 minutes	1·40

ANTIMONY SULPHIDE

Hydrogen sulphide was passed through a solution of pottassium antimony tartrate. An orange-coloured sol of antimony sulphide was obtained which was freed from sulphuretted hydrogen and tartrate by dialysis at the ordinary temperature for ten days.

TABLE V

Concentration of sol = 0·53 grm. of Sb_2S_3 per litre
Volume of the mixture = 10 c.c.
Amount of sol taken = 1 c.c.
Time allowed for coagulation = 1 hour

Electrolyte used.	Amount needed (in c. c.) for coagulation.	
	Unexposed sol.	Sol exposed for 27 hours and 20 minutes.
KCl N	0·55 c.c.	0·80
$BaCl_2$ N/40	0·80 c.c.	1·60 c.c.

The sol coagulated after 52 hours and 50 minutes' exposure in a glass vessel, so that the conductivity and extinction coefficients could not be noted.

In the following experiments antimony and arsenious sulphide sols were exposed to light in silica vessels.

ANTIMONY SULPHIDE

The sol was prepared in the usual way but was not subjected to dialysis. The sol was transparent.

TABLE VI

Concentration of sol = 0·9430 grm. of Sb_2S_3 per litre
Volume of the mixture = 5 c.c.
Amount of sol taken = 1 c.c.
Time allowed for coagulation = 1 hour

Date.	Electrolyte used KCl N/10	Amount needed for coagulation.
12-2-29	2·2 c.c.	Sol kept in the dark.
	2·2 c.c.	Sol exposed to light from a 1000-watt-lamp for 3 hours.
	2·5 c.c.	Sol exposed to light from a 1000-watt-lamp for $4\frac{1}{2}$ hours.
	2·5 c.c.	Sol exposed to sunlight for 3 hours.
13-2-29	2·4 c.c.	Sol kept in the dark.
	2·4 c.c.	Sol exposed to light from a 1000-watt-lamp for 9 hours.
19-2-29	2·5 c.c.	Sol exposed to light from a 100-watt-lamp for 9 hours.
	2·2 c.c.	Sol exposed to sunlight for 13 hours 18 minutes.

ELECTRIC CONDUCTIVITY.

Sp. conductivity for sol kept in the dark.	Sp. conductivity for sol exposed to light.
0·000627	0·000629 Sol exposed to light from a 1000-watt-lamp for 3 hours.
	0·000632 Sol exposed to light from a 1000-watt-lamp for $4\frac{1}{2}$ hours.
	0·000653 Sol exposed to light from a 1000-watt-lamp for 9 hours.
	0·000682 Sol exposed to sunlight for 3 hours.
	0·001490 Sol exposed to sunlight for 13 hours and 18 minutes.

EXTINCTION COEFFICIENTS.

Sol taken.	Extinction coefficients.
Sb_2S_3 kept in the dark	0·60
Sb_2S_3 exposed to light from a 1000 watt-lamp for $4\frac{1}{2}$ hours	0·49
Sb_2S_3 exposed to light from a 1000 watt-lamp for 9 hours	0·52

ARSENIOUS SULPHIDE

The sol was prepared by passing hydrogen sulphide in a concentrated solution of Arsenious oxide in water. In order to free it from hydrogen sulphide a current of hydrogen was passed through it.

TABLE VII

Concentration of the sol = 52·79 grms. As_2S_3 per litre
Volume of mixture = 5 c.c.
Amount of sol taken = 1 c.c.
Time allowed for coagulation = 1 hour

Electrolyte used.	Amount needed for coagulation.
KCl N/10	3·10 c.c. for sol kept in the dark 3·20 c.c. ,, ,, exposed for $\frac{1}{2}$ hour 3·10 c.c. ,, ,, ,, ,, 1 ,, 3·05 c.c. ,, ,, ,, ,, 2 hours 3·00 c.c. ,, ,, ,, ,, 3 ,, 2·90 c.c. ,, ,, ,, ,, 4 hours and 45 minutes

ELECTRIC CONDUCTIVITY.

Sp. conductivity for sol. kept in the dark.	Sp. conductivity for sol. exposed to light.
0·000303	0·000364 Sol exposed for 1 hour 0·000439 ,, ,, ,, 2 hours 0·000502 ,, ,, ,, 3 ,,

EXTINCTION COEFFICIENTS.

Sol taken.	Extinction coefficients.
As_2S_3 kept in the dark ...	1·73
As_2S_3 exposed for half an hour to sunlight ...	1·48
As_2S_3 exposed for 3 hours to sunlight ...	1·83

URANIUMFERROCYANIDE

Solutions of Uranium nitrate and potassium ferrocyanide were mixed at the ordinary temperature and a brownish red sol of Uraniumferrocyanide was obtained in presence of an excess of potassium ferrocyanide. The sol was dialysed for 12 days in order to free it from potassium ferrocyanide.

TABLE VIII

Concentration of sol = 4·0 grms. uraniumferrocyanide per litre
Volume of the mixture = 5 c.c.
Amount of sol taken = 1 c.c.
Time allowed for coagulation = 1 hour

Electrolyte used.	Amount in c.c. needed for coagulation.	
	Unexposed sol.	Exposed for 10 hrs. 20 minutes.
KCl N/2·5	2·5 c.c.	2·3 c.c.
$BaCl_2$ N/40	1·9 c.c.	1·7 c.c.

Completely coagulated after 33 hours 15 minutes' exposure to sunlight. Hence electric conductivity could not be determined.

EXTINCTION COEFFICIENTS.

Sol taken.	Extinction coefficients.
Uraniumferrocyanide kept in the dark ...	3·8
Uraniumferrocyanide exposed for 23 hours and 5 minutes ...	2·2

In the following experiments uranium and cupric ferrocyanide sols were exposed to sunlight in silica vessels.

URANIUMFERROCYANIDE

TABLE IX

Concentration of the sol = 0·955 grm. of uraniumferrocyanide per litre
Volume of the mixture = 5 c.c.
Amount of the sol = 1 c.c.
Time allowed for coagulation = 1 hour.

Date.	Electrolyte.	Amount needed for coagulation.
10-1-29	KCl N/10	1·6 c.c. for sol kept in the dark.
		1·55 c.c. „ „ exposed to light for $2\frac{1}{2}$ hours
		1·50 c.c. „ „ „ „ „ „ 3 „
15-1-29		1·40 c.c. „ „ kept in the dark
		1·60 c.c. „ „ exposed to light for 1 hour

The sol coagulated on further exposure to light so that the electric conductivity and extinction coefficient results could not be noted.

CUPRICFERROCYANIDE

TABLE X

Concentration of the sol = 22·58 grms. of cupricferrocyanide per litre.

Volume of the mixture = 5 c.c.
Amount of sol taken = 1 c.c.

Date.	Electrolyte used.	Amount needed for coagulation.
2-2-29	KCl N/10	0·65 c.c. for sol kept in the dark
		0·70 c.c. ,, sol exposed to light for one hour
		0·85 c.c. ,, sol exposed to light for two hours
		0·75 c.c. ,, sol exposed to light for three hours
28-2-29		0·85 c.c. ,, kept in the dark
		0·80 c.c. ,, exposed to light for 25 hours

ELECTRIC CONDUCTIVITY.

Sp. conductivity for sol. kept in the dark.	Sp. conductivity for sol. exposed to sunlight.
0·000184	0·000134 for sol exposed to light for one hour
	0·000165 for sol exposed to light for two hours
	0·000168 for sol exposed to light for three hours

EXTINCTION COEFFICIENTS.

Sol taken.	Extinction coefficients.
Cupricferrocyanide kept in the dark ...	2·98
Cupricferrocyanide exposed for two hours	2·48
Cupricferrocyanide exposed for $7\frac{1}{2}$ hours	3·08

The experimental results on the influence of light on colloids show that sols of $Sn(OH)_4$, $Th(OH)_4$, $Al(OH)_3$ and HgS become unstable towards electrolytes on exposure to light. The electric conductivity of these sols increases on exposure.

On the other hand, sols of Sb_2S_3, As_2S_3, uranium-ferrocyanide and cupricferrocyanide when exposed to light for a short time become stable but on longer exposure they become unstable towards electrolytes. On further exposure Sb_2S_3, HgS, and uraniumferrocyanide coagulate completely.

In Papers, published from these laboratories, we have shown that sols of ferric hydroxide, aluminium hydroxide, stannic hydroxide, etc., become more conducting, less viscous and less stable towards electrolytes on ageing. Our results show that the effect of light is of the same type as that of ageing on the hydroxide sols. We have also shown that sols of arsenious sulphide, antimony sulphide and cupric and uranium ferrocyanides are appreciably hydrolysed in aqueous solutions and this hydrolysis makes the sol more stable towards electrolytes. Moreover we have also shown that on ageing the sols become more hydrolysed and more stable towards electrolytes up to a limiting value. The foregoing results show that under the influence of light these sols become more hydrolysed and more stable towards electrolytes when exposed for a short time specially in silica vessels. On longer exposure the sols become unstable towards electrolytes. This behaviour is due to the fact that in the cases of arsenious and antimony sulphides, the stabilising electrolyte hydrogen sulphide is oxidised by air. Moreover several of the sulphides are also oxidised to sulphur and thionic compounds. In the case of the ferrocyanides the stabilising ferrocyanide ions decompose on longer exposure making the sols unstable towards electrolytes. The first effect of light is to increase the degree of hydrolysis of the sulphide, the ferrocyanides and

other easily hydrolysable also and hence increase their stability towards electrolytes. This effect is more than counterbalanced by the oxidation or the decomposition of the stabilising ion on longer exposure rendering the sols unstable towards electrolytes and their subsequent coagulation on long exposure.

Our experimental results show that in general the effect of light on sols is in the same direction as that of ageing. The light effect seems more pronounced than the time effect.

The measurements of extinction coefficients of sols as recorded in the Paper do not warrant any definite conclusion regarding the absorptive power of the exposed and unexposed sols. Exposed sols have greater absorption capacity than the unexposed one. Further work in this line is in progress in this laboratory.

SUMMARY

1. Sols of stannic hydroxide, aluminium hydroxide, thorium hydroxide and mercuric sulphide become unstable towards mono- and bivalent ions on exposure to light.

2. Arsenious sulphide, antimony sulphide, uranium ferrocyanide and cupric ferrocyanide when exposed to light for a short time become stable in their coagulation by electrolytes; but, on longer exposure they become unstable.

3. On prolonged exposure sols of antimony sulphide, uranium ferrocyanide and mercuric sulphide coagulate completely.

4. Exposed sols of stannic hydroxide, aluminium hydroxide, thorium hydroxide, mercuric sulphide, antimony sulphide and arsenious sulphide are more electrically conducting than the respective unexposed ones.

5. Measurements of extinction coefficients of sols show that in some cases the exposed sols show more absorption of light than the unexposed ones. No general conclusions can be arrived at regarding light absorption of sols on exposure to light.

OXIDATION OF CARBOHYDRATES, FATS AND NITROGENOUS SUBSTANCES BY HYDROGEN PEROXIDE AND FERRIC SALTS

BY

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In a series of publications from this laboratory the slow and induced oxidation of organic and inorganic substances has been investigated (J. Phys. Chem., 1925, *29*, 376, 799 ; 1926, *30*, 939 ; 1924, *28*, 943 ; 1928, *32*, 1263, 1663) and the mechanism of the oxidation in the animal body has been imitated. It is well known that the edible substances like carbohydrates, fats and proteins are very readily oxidised in the body whereas they are oxidised under difficulty in the laboratory. But in the papers referred to, it has been shown that the edible substances can be oxidised in the laboratory also at the ordinary temperature by passing air, if inductors like ferrous hydroxide, cerous hydroxide, sodium sulphite, etc., are present. It is interesting to note that these edible substances can be oxidised by passing air through them if they are exposed to sunlight even without any catalyst. In each of these oxidations, complete oxidation to carbon-dioxide takes place. Even complex substances like butter, lecithin, milk, egg-white, egg-yellow, cholesterol, etc., are oxidised almost quantitatively into carbon dioxide and water.

We know that a deviation from this rule is found in animal bodies during certain diseases and also during fasting. In diabetic patients, glucose is practically not oxidised and acetone bodies are found in the organism. In normal health, the heat and energy of the body is derived from the simulta-

neous and slow combustion of carbohydrates, fats and nitrogenous substances. But in diabetes, the carbohydrates are not oxidised. Hence, in order to supply the necessary heat and energy to the body, the oxidation of fats and nitrogenous substances has to become more rapid than in normal health. It appears therefore that when the oxidation of fats and nitrogenous substances is more rapid than in normal health, acetone bodies are likely to be formed. The acetone bodies are also formed during long fasting. In starvation the necessary amount of heat and energy is obtained from the body itself and the glycogen store in the body is first oxidised. When the glycogen is exhausted, in order to bring in the same amount of energy, a more rapid oxidation of the body tissues and body fats takes place with the result that acetone bodies are formed. Dakin (J. Biol. Chem., 1908, *4*, 77) had shown that ammonium butyrate, on being oxidised with hydrogen peroxide, produced acetone, acetoacetic acid, acetaldehyde, etc. As hydrogen peroxide is believed to be a more rapid oxidising agent than air, it was thought desirable to investigate the oxidation of edible substances with hydrogen peroxide and ferric salt as an accelerator, in order to find out the nature of the change occurring if the oxidation in animal body becomes more rapid than in normal health. In the present paper, the oxidation of edible substances either occurring singly or in mixtures, by hydrogen peroxide and ferric salt has been investigated and our results have thrown considerable light in the metabolism in diabetes and in starvation.

In several publications from these laboratories we have proved that the slow oxidation of substances can be retarded by another reducing agent which usually undergoes slow oxidation along with the primary change (Proc. Akad. Wet. Amsterdam, *29*, 1023 (1921); z. anorg. chem., *144*, 289 (1925); cf. Moureu & Dufraisse Compt. Rend., *179*, 237 (1924). Thus we have shown that the oxidation of sodium

sulphite by air is markedly retarded by sodium arsenite which undergoes oxidation by air in presence of sodium sulphite. Similarly we have proved that the slow oxidation of carbohydrates by air is retarded by fats and nitrogenous substances and the oxidation of fats and nitrogenous substances is retarded by the presence of glucose. In the present paper, we have also investigated the retardation of the oxidation of one edible substance by hydrogen peroxide in presence of another.

EXPERIMENTAL

10 c.c. of 1% solution of carbohydrates, fats and nitrogenous substances were mixed with 10 c.c. of hydrogen peroxide (Merck's 12 vol.) and 1 c.c. of ferric sulphate solution containing 0·005 grm. of iron. The mixture was taken in a flask and kept at 37° at the body temperature in a thermostat for 5 hours. The products were carefully distilled by heating the mixture for forty-five minutes over the direct flame of a bunsen burner. The distillate and the residue were treated with the following reagents, Fehling's solution, Schiff's reagent, mercuric chloride, ammoniacal silver nitrate, bromine water, calcium chloride and iodine with caustic soda. The smell of the product was also noted. The results are tabulated in Table I.

TABLE I

Reactions with various reagents after oxidising different organic substances with H_2O_2.

Reagents	Potassium Stearate 0·1 gm.		Potassium oleate 0·1 gm.		Potassium palmitate 0·1 gm.		Glucose 0·1 gm.		Starch 0·1 gm.		Alanine 0·1 gm.		Lactic acid 0·1 gm.	
	Distillate	Residue	Distillate	Residue	Distillate	Residue	Distillate	Residue	Distillate	Residue	Distillate	Residue	Distillate	Residue
Fehling's solution	Reduced	...	Reduced	...	Reduced	...	Reduced	Reduced	Reduced	Reduced	Reduced	...	Reduced	...
Schiff's reagent	...	...	...	...	...	...	...	...	...	...	Pink	...	Pink	...
Mercuric chloride	...	Reduced	Reduced	Reduced	...	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced
Am. silver nitrate	Reduced	...	Reduced	...	Reduced	...	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced
Bromine water	Decolorised	...	Decolorised	...	Decolorised	...	Decolorised	...	...	...	Decolorised	...	Decolorised	...
Calcium chloride	...	White ppt.	...	White ppt.	...	White ppt.	...	...	...	...	...	...	...	...
Alkaline Iodine	Iodoform	...	Iodoform	...	Iodoform	...	...	...	...	...	Iodoform	...	Iodoform	...
Smell	Acrolein	...	Acrolein	...	Acrolein	...	...	...	...	...	Acetaldehyde	...	Acetaldehyde.	...

In the cases of potassium palmitate, stearate and oleate, the smell of acrolein could be distinctly perceived in the distillate and in the last case, the smell was very strong. In the case of lactic acid and alanine, a distinct smell of acetaldehyde was found. But in the majority of cases the products could not be definitely identified because they contain numerous ingredients. The volatile aldehydic and ketonic products were estimated in the following way.

The above mixture of the edible substances, hydrogen peroxide and ferric salt, after keeping at 37° for 5 hours were taken out and the volume was made up to 50 c.c. in every case. They were then distilled slowly for 45 minutes in the usual way and the distillate absorbed in water. 10 c.c. of N/10 caustic soda and 10 c.c. of N/10 Iodine solution were then added to the distillate and kept aside for ten minutes. A portion of the iodine was taken up by the aldehydic products and iodoform was produced in appreciable quantities in the cases of fats, and some nitrogenous substances. The solution was acidified with 10 c.c. of N/10 sulphuric acid and the liberated iodine was titrated against N/10 sodium thiosulphate, thus giving the amount of iodine taken up by the volatile products. The results are given in the Tables Nos. II, III, IV, and V.

TABLE II

Carbohydrates + Hydrogen peroxide + $Fe_2(SO_4)_3$.

Total volume 50 c.c.

Carbo-hydrates.	Amt. of carbo-hydrates.	Amount of H_3O_3	Amt. of Iron.	Temp.	Time.	Amt. of N/10 iodine absorbed.
Glucose	0·1 grm.	10 c.c.	0·005 gm.	37°C	5 hrs.	2·6 c.c.
Fructose	do.	do.	do.	do.	do.	3·2 c.c.

Carbo-hydrates.	Amt. of carbo-hydrates.	Amount of H_2O_2	Amt. of Iron.	Temp.	Time.	Amt. of N/10 iodine absorbed.
Lactose	0·1 grm.	10 c.c.	5·00 gm.	37° C	3 hrs.	3·8 c.c.
Arabinose	do.	do.	do.	do.	do.	3·5 c.c.
Galactose	do.	do.	do.	do.	do.	4·2 c.c.
Maltose	do.	do.	do.	do.	do.	6·0 c.c.
Sucrose	do.	do.	do.	do.	do.	1·7 c.c.
Starch ...	do.	do.	do.	do.	do.	2·6 c.c.
Dextrin ...	do.	do.	do.	do.	do.	3·3 c.c.
Inulin ...	do.	do.	do.	do.	do.	3·3 c.c.
Glycogen	do.	do.	do.	do.	do.	4·2 c.c.

Table III

Nitrogenous substances + Hydrogen peroxide + $Fe_2(SO_4)_3$.

Total volume 50 c.c.

Nitrogenous substances.	Amt. of substance.	Amt. of H_2O_2.	Amount of Iron.	Temp.	Time.	Amt. of N/10 iodine absorbed.
Alanine ...	0·1 grm.	10 c.c.	0·005grm	37°C	5 hrs.	3·7 cc.
Glycine ...	do.	do.	do.	do.	do.	3·4 cc.
Egg-white	do.	do.	do.	do.	do.	3·8 cc.
Egg-yellow	do.	do.	do.	do.	do.	3·3 cc.
Urea ...	0·5 grm.	do.	do.	do.	do.	Nil.

TABLE IV

Fatty substances + Hydrogen peroxide + $Fe_2(SO_4)_3$.

Total volume 50 c.c.

Fatty substances.	Amt. of substance.	Amt. of H_2O_2.	Amount of Iron.	Temp.	Time.	Amt. of N/10 iodine absorbed.
Potassium oleate	0·1 gm.	10 c.c.	0·005 gm	37°C	5 hrs.	4·9 c.c.
Potassium stearate	do.	do.	do.	do.	do.	2·4 c.c.
Potassium Palmitate	do.	do.	do.	do.	do.	4·6 c.c.
Butter ...	0·462gm.	20 c.c.	do.	do.	24 hrs.	5·6 c.c.

TABLE V

Cholesterol + Hydrogen peroxide + $Fe_2(SO_4)_3$.

Volume 50 c.c.

	Amt. of the substance.	Amt. of H_2O_2.	Amount of Iron.	Temp.	Time.	Amt. of N/10 iodine absorbed.
Cholesterol	0·5 gm.	20 c.c.	0·005gm.	37°C	24 hrs.	2·6 c.c.

The mutual retardation in the oxidation of the food materials with hydrogen peroxide and ferric salt was measured in the following way. 10 c.c. each of carbohydrates and nitrogenous substance were mixed and 20 c.c. of H_2O_2 were added. The temperature was kept at 37° but the time of the reaction was extended to 25 hours. After the reaction, the volume was made up to 100 c.c. and the distillation carried on for about an hour and a quarter. The distillate was absorbed in water and 20 c.c. of N/10 NaOH and 20 c.c. of N/10 Iodine were added and the residual iodine estimated in the usual way. Similar treatment

was made in the mixtures of carbohydrates and fats and fats and nitrogenous substances. In the mixture of all the three varieties, the amount of hydrogen peroxide was increased to 20 c.c., and the time was extended to 48 hours. The results are tabulated in TablesVI, VII, VIII, IX, X and XI.

TABLE VI

Carbohydrates + Nitrogenous substances + H_2O_2 + $Fe_2(SO_4)_3$.

Amt. of H_2O_2—20c.c. Carbohydrates—0·1 gm. Nitrogenous matter—0·1 gm.

Carbohydrates.	Alanine (3·7)	Glycine (3·4)	Urea	Egg-white (3·8)	Egg-yellow (3·3)
Glucose (2·6)	5·3 c.c.	3·6 c.c.	2·5 c.c.	3·1 c.c.	1·9 c.c.
Fructose (3·2)	3·7 ,,	2·1 ,,	...	2·3 ,,	3·2 ,,
Lactose (3·8)...	4·0 ,,	3·6 ,,	...	1·6 ,,	6·7 ,,
Arabinose (3·5)	1·9 ,,	3·7 ,,	...	4·4 ,,	4·8 ,,
Galactose (4·2)	4·7 ,,	4·4 ,,	...	4·8 ,,	5·1 ,,
Maltose (6·0)...	5·2 ,,	4·2 ,,	...	1·9 ,,	6·2 ,,
Sucrose (1·7)...	3·2 ,,	2·9 ,,	...	2·1 ,,	6·2 ,,
Starch (2·6) ...	1·8 ,,	4·8 ,,	...	3·3 ,,	3·7 ,,
Inulin (3·3)	2·7 ,,	3·1 ,,	...	2·7 ,,	3·5 ,,
Dextrin (3·3) ...	4·8 ,,	2·4 ,,	...	2·5 ,,	5·3 ,,
Glycogen (4·2)	3·9 ,,	4·3 ,,	...	2·6 ,,	5·9 ,,

Iron—0·005 grm., Temperature—37°, Time 24—hours. Total volome—100 c.c. In the experiment with egg, the amount of egg was 0·5 grm. The figures in the bracket in the above table show the amount of N/10 Iodine absorbed by the single substance after oxidation. The other figures indicate the amouut of N/10 Iodine absorbed by the mixtures after oxidation.

TABLE VII

Carbohydrates + Fats + Hydrogen peroxide + $Fe_2(SO_4)_3$.

Amount of H_2O_2 — 20 c.c., Carbohydrates—0·1 grm. Fats—0·1 grm. (except butter 0·462 grm. of which was taken). Iron—0·005 grm. Temperature—37°C. Time—25 hours (except in the case of mixture with butter where the time was extended to 72 hours.) Total volume 100 cc.

	Potassium oleate (4·9)	Potassium stearate (2·4)	Potassium palmitate (4·6)	Butter (5·6)
Glucose (2·6) ...	5·2 c.c.	2·6 c.c.	4·7 c.c.	6·3 c.c.
Fructose (3·2) ...	1·1 ,,	1·9 ,,	4·6 ,,	3·9 ,,
Lactose (3·8) ...	4·6 ,,	4·6 ,,	3·8 ,,	5·9 ,,
Arabinose (3·5) ...	5·5 ,,	3·3 ,,	2·1 ,,	6·7 ,,
Galactose (4·2) ...	5·7 ,,	4·3 ,,	5·3 ,,	5·6 ,,
Maltose (6·0) ...	5·7 ,,	2·0 ,,	4·5 ,,	6·0 ,,
Sucrose (1·7) ...	2·2 ,,	1·3 ,,	1·7 ,,	5·1 ,,
Starch (2·6) ...	2·5 ,,	1·1 ,,	2·2 ,,	4·9 ,,
Inulin (3·3) ...	6·3 ,,	5·6 ,,	6·0 ,,	6·0 ,,
Dextrin (3·3) ...	3·2 ,,	3·6 ,,	3·6 ,,	3·2 ,,
Glycogen (4·2) ...	6·7 ,,	6·4 ,,	6·0 ,,	3·7 ,,

The figures in the bracket in the above table show the amount of N/10 Iodine absorbed by the single substance after oxidation. The other figures indicate the amount of N/10 Iodine absorbed by the mixtures after oxidation.

TABLE VIII

Fats + Nitrogenous substances + Hydrogen peroxide + $Fe_2(SO_4)_3$.

Amount of H_2O_2—20 c.c. Fats—0·1 grm. (except butter 0·462 grm. of which was taken). Nitrogenous substances—0·1 grm. (except egg 0·5 grm. of which was taken). Iron 0·005 grm. Temperature—37°C. Time—25 hours (except in the case of mixture with butter where the time was extended to 72 hours). Total volume 100 c.c.

	Alanine (3·7)	Glycine (3·4)	Urea.	White-egg (3·8)	Egg-yellow (3·3)
Potassium oleate (4·9) ...	4·6 c.c.	2·3 c.c.	2·8 c.c.	3·3 c.c.	6·6 c.c.
Potassium stearate (2·4) ...	2·1 ,,	3·5 ,,	2·2 ,,	3·8 ,,	6·9 ,,
Potassium palmitate (4·6) ...	4·1 ,,	3·1 ,,	1·2 ,,	6·4 ,,	6·7 ,,
Butter (5·6) ...	4·4 ,,	6·7 ,,	..	4·4 ,,	6·5 ,,

The figures in the bracket in the above table show the amount of N/10 Iodine absorbed by the single substance after oxidation. The other figures indicate the amount of N/10 iodine absorbed by the mixture after oxidation.

TABLE IX

Cholesterol + Fats + Hydrogen peroxide + $Fe_2(SO_4)_3$.

Amount of H_2O_2—30 c.c. Fats—0·1 grm. Cholesterol —0·5 grm. Iron—0·005 grm. Temperature—37°C. Time—60 hours. Total volume—100 c.c.

	Potassium stearate (2·4)	Potassium palmitate (4·6)	Fotassium oleate (4·9)
Cholesterol (2·6)	4·0	5·6	4·7

The figures in the bracket in the above table show the amount of N/10 Iodine absorbed by the single substance after oxidation. The other figures indicate the amount of N/10 Iodine absorbed by the mixtures after oxidation.

TABLE X

Carbohydrates + Fats + Nitrogenous substances + Hydrogen peroxide + $Fe_2(SO_4)_3$.

Carbohydrates, Oleate, Stearate, Palmitate	0·1 grm. each.	Butter 0·462 grm. Egg-white Egg-yellow	0·5 grm. each

H_2O_2—30 c.c. Iron—0·005 grm. Temperature—37°C. Time—50 hours. Total volume—100 c.c.

Mixtures.	Amount of N/10 iodine absorbed.
Glycogen (4·2) + Alanine (3·7) + Oleate (4·8) ...	6·0 c.c.
,, ,, + Stearate (2·5) ..	4·8 ,,
,, ,, + Palmitate (4·4) ...	6·1 ,,
Butter (5·6) + Egg-white (3·8) + Glucose (2·6) ...	5·3 ,,
,, ,, + Sucrose (1·7) ...	3·1 ,,
,, ,, + Starch (2·6) ...	5·2 ,,
,, ,, + Inulin (3·3) ...	3·6 ,,
,, ,, + Dextrin (3·3) ...	5·6 ,,
,, ,, + Glycogen (4·2) ...	3·8 ,,
Butter (5·6) + Egg-yellow (3·3) + Glucose (2·6) ...	8·3 ,,
,, ,, + Sucrose (1·7) ...	7·3 ,,
,, ,, + Starch (2·6) ...	6·6 ,,
,, ,, + Inulin (3·3) ...	9·6 ,,
,, ,, + Dextrin (3·3) ...	8·6 ,,
,, ,, + Glycogen (4·2) ...	6·8 ,,

The figures in the bracket in the above table show the amount of N/10 Iodine absorbed by the single substance after oxidation. The other figures indicate the amount of N/10 Iodine absorbed by the mixtures after oxidation.

TABLE XI

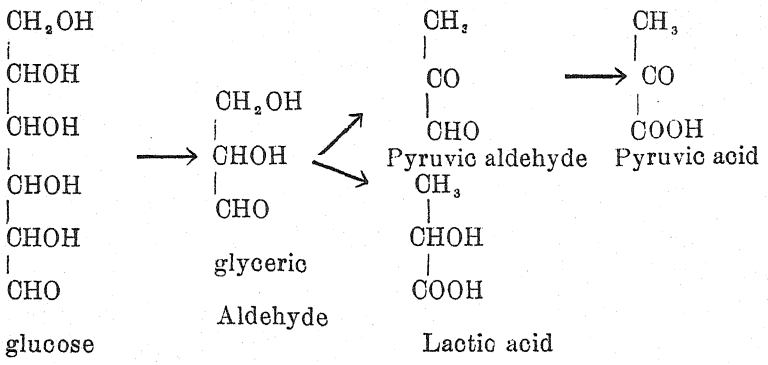
Carbohydrates+Fats+Hydrogen peroxide+$Fe_2(SO_4)_3$ (when the amount of carbohydrate was increased).

Amount of H_2O_2—75 c.c., Carbohydrates—0·5 grm. Oleate—0·2 grm. Butter—0·925 grm. Iron—0·005 grm. Temperature—37°C. Time—50 hours. Total volume—150 c.c.

Mixtures.			Amount of N/10 Iodine.
Glucose (12 c.c.)	+Oleate (10·1)	...	8·2 c.c.
Starch (12·2)	,,	...	5·3 c.c.
Sucrose (9)	,,	...	5·1 c.c.
Dextrin (15)	,,	...	7·4 c.c.
Glycogen (19·8)	,,	...	6·9 c.c.
Inulin (15·2)	,,	...	8·4 c.c.
Glucose (12)	+Butter (11·5)	...	9·9 c.c.
Starch (12·2)	,,	...	5·7 c.c.
Sucrose (9)	,,	...	5·5 c.c.
Dextrin (15)	,,	...	7·8 c.c.
Glycogen (19·8)	,,	...	8·3 c.c.
Inulin (15·2)	,,	...	9·8 c.c.

The figures in the bracket in the above table show the amount of N/10 Iodine absorbed by the single substance after oxidation. The other figures indicate the amount of N/10 Iodine absorbed by the mixtures after oxidation.

A glance at the above table shows that in each case a certain amount of iodine has been taken up by the volatile products showing that substances of aldehydic and ketonic nature have been produced during the reaction. The amount of the substances of aldehydic or ketonic nature formed is greatest in the case of fats, specially butter and least in the case of carbohydrates, specially sucrose. It is interesting to note that not only do the fats and nitrogenous substances produce compounds of aldehydic or ketonic nature but even the carbohydrates produce substances of this type. The explanation is not far to seek. The carbohydrates on oxidation give rise to glyceric aldehyde, pyruvic aldehyde, pyruvic acid and lactic acid in the following way:—



Lactic acid very easily oxidises to acetaldehyde (cf., G.B. Ray, Journal Gen. Physiol., 1924, *6*, 509, 528).

The higher fatty acids gradually oxidise into the lower fatty acids due to β-oxidation (Beitr. Z. Chem. Physiol., 1908, *11*, 411) and ultimately to butyric acid from which the production of acetone bodies can be explained. The amino acids on oxidation give the hydroxy acids, the lower acids and ultimately aldehydic and ketonic products. It has been known from a long time that alanine can produce acetaldehyde on oxidation, and hence if the proteins hydrolyse to the stage of amino acids, as it usually does in the

body, the production of alanine or like product can explain the production of acetone bodies from the proteins.

It is interesting to note from the foregoing data that in mixtures, the amount of iodine absorbed by the volatile product after oxidation with hydrogen peroxide is remarkably reduced showing that the amount of the substances of aldehydic nature is decreased. The retardation is greatest in the case of starch and sucrose. Table XI shows that if the amount of carbohydrates be increased the amount of aldehydic product is still more decreased. Hence the presence of carbohydrates decreases the amount of acetone bodies formed by the oxidation of fats by hydrogen peroxide and ferric salts.

It appears therefore that some methods of treatment in diabetes are faulty. A school of medical men prefer the administration of fats to diabetic patient arguing that as glucose cannot be oxidised in the body, the required energy must be supplied by fat. But our results show that in the absence of glucose, fats cannot be oxidised in the normal manner. On the contrary, they produce poisonous bodies like acetone, diacetic acid, etc. So the more the patient gets fats, the more is the quantity of acetone bodies formed and thus the end of the patient is likely to be hastened. Similar arguments can be brought against those who prefer protein ingestion. In order that the oxidation in the animal body should proceed in the normal manner, glucose must be oxidised. So the treatment of diabetic patients should depend upon the fundamental principle that the glucose must be more largely oxidised.

It is well-known that by the injection of insulin, acetone bodies disappear from the body. This is due to the fact that by the presence of insulin, the glucose which was passing unoxidised from the diabetic system is oxidised and the oxidation of the glucose leads to the more complete though more slow oxidation of fats.

SUMMARY

1. Sugars, starch, glycogen, dextrin, inulin, nitrogenous substances like alanine, egg-white, egg-yellow, etc., fats like butter, oleate, stearate, palmitate, etc., were oxidised with hydrogen peroxide and ferric salt and the volatile aldehydic or ketonic products were estimated by the amount of iodine taken up by them in the formation of iodoform. It has been proved that not only the fats but the carbohydrates and nitrogenous substances have the property of producing acetone bodies.

2. The oxidation of the mixtures of fats and proteins, fats and carbohydrates, carbohydrates and proteins and the mixture of all the three varieties by hydrogen peroxide and ferric salt has been investigated and it has been shown that in mixtures, the amount of acetone bodies formed are markedly decreased. Thus the oxidation of fat is retarded by proteins and carbohydrates, the oxidation of protein is retarded by carbohydrates and fats and the oxidation of carbohydrates is retarded by fats and proteins.

3. It has also been shown that in the oxidation of the mixtures of carbohydrates and fats, with hydrogen peroxide and ferric salt, with the increase of the amount of carbohydrates, the amount of acetone bodies is decreased.

4. The experimental results have thrown considerable light on the metabolism in the animal body in normal health, in diabetes and in starvation.

5. It seems that the disappearance of acetone bodies from the diabetic organism due to the injection of insulin is caused by the increased oxidation of the negative catalyst glucose.

INVESTIGATION ON THE PRODUCTS OBTAINED BY EXPOSING OILS AND CARBOHYDRATES TO SUNLIGHT IN PRESENCE OF AIR

BY

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In a recent publication from this laboratory (Palit and Dhar, *Jour. Phys. Chem.*, 1928, 32, 1663) it has been shown that the edible products like carbohydrates, fats and nitrogenous substances can be oxidized by passing air through their solutions in presence of sunlight. These results are interesting in view of the observations made by different workers who are of opinion that inert substances on being irradiated with quartz-mercury vapour lamp are endowed with antirachitic properties. These investigators are of opinion that vitamins are synthesized by the ultra-violet radiations when edible substances are irradiated. The present work was undertaken in order to investigate whether actually vitamins are synthesized or some other type of activation takes place. In a recent paper from this laboratory the view had been advanced that when substances like cholesterol, olive oil, etc., are exposed to light in presence of air, peroxides are formed and these induce the oxidation of food-materials mixed with them. Hence the antirachitic and beneficial properties of substances not containing the necessary vitamins are due to the presence of peroxides which help the oxidation of the food-materials

in the body. Substances can acquire antirachitic properties when exposed to light only in presence of air or oxygen. It will be observed that this view is supported by the following observations :—

EXPERIMENTAL

Equal quantities of different oils were taken in small beakers containing 25 c.c. of water and they were exposed to sunlight for 5 hours. A current of air free from carbon dioxide was constantly being passed through the oils. After the exposure, an excess of potassium iodide solution was added to the mixture and the mixture was acidified. The liberated iodine was titrated against N/100 sodium thiosulphate solution with starch as an indicator. The results are shown in Table I.

TABLE I

Oils.	Botanical name.	Amount taken in c.c.	Amount of iodine liberated in terms of c.c. of N/100 thiosulphate.
Olive oil ...	*Olea europœa* ...	3	3·20
Mustard oil..	*Brassica competris*	3	1·4
Cocoanut oil	*Cocos nucifera* ...	3	1·00
Mahua oil ..	*Bassia latifolia* ...	3	1·20
Castor oil ...	*Ricinus communis*	3	0·95
Til oil ...	*Sesamum indicum*	3	1·2
Linseed oil ...	*Linum usitatissimum.*	3	0·80

These experiments were repeated and almost the same results were obtained showing that the results are reproduceable.

Not only these oils but also butter and some carbohydrates behaved in the same manner after being exposed

to sunlight. The amount of iodine liberated by them from an acid solution of potassium iodide is given in Table II.

TABLE II

Substances.	Amount taken in grammes.	Amount of iodine liberated in terms of c.c. of N/100 thiosulphate.
Butter ...	0·92	2·25
Starch ...	0·10	0·20
Glycogen ...	0·10	0·05
Dextrin ...	0·10	0·45
Glucose ...	0·10	0·05

The irradiated substances had the property of oxidizing other substances when mixed with them. The extent to which they oxidized glucose was determined in the following way :—Ten c.c. of 1 per cent glucose solution was mixed with the oils after irradiation in the presence of air and the mixture was kept at 40°C. in a thermostat for 18 hours. The mixture was filtered when the oil remained on the filter paper. The oil was washed well with warm water. In each case a blank experiment was also done by keeping 10 c c. of the same glucose solution in contact with the same quantity of unexposed oils, the temperature and time being the same. This brought the possible experimental error to a minimum. The solution containing glucose was precipitated with Fehling's solution and the cupric oxide obtained on ignition was weighed. The oil which remained on the filter paper was taken out and again an acid solution of potassium iodide was added to it and the liberated iodine titrated against N/100 sodium thiosulphate. The results are given in Table III.

TABLE III

Substance.	Amount of oil in c.c.	Amount of N/100 iodine in c.c. liberated before the oxidation of glucose.	Amount of CuO in grms. (blank).	Amount of CuO in grms. (after oxidation).	Percentage oxidized.	Amount of N/100 iodine in c.c liberated after oxidation of glucose.
Mustard oil	3	1·50	0·2183	0·2071	5·1	0·20
Cocoanut oil	3	1·05	0·2169	0·2108	2·9	0·35
Mahua oil	3	1·20	0·2222	0·2132	3·9	0·15
Castor oil	3	0·95	0·2166	0·2084	3·8	...
Til oil ...	3	1·25	0·2217	0·2178	1·7	0·85
Linseed oil	3	1·00	0·2177	0·2140	1·2	0·70

The experiments on oxidation were repeated several times and fairly reproduceable results were obtained as shown in Table IV. On exposing these oils to sunlight without passing air, similar results were obtained though the amount of oxidation is much less as shown in Table V.

TABLE IV

Substance.	Amount of oil in c.c.	Amount of N/100 iodine in c.c. liberated before the oxidation of glucose.	Amount of CuO in grms. (blank).	Amount of CuO in grms. (after oxidation).	Percentage oxidized.	Amount of N/100 iodine in c.c. liberated after oxidation of glucose.
Mustard oil	3	1·5	0·2331	0·2202	5·5	0·10
Cocoanut oil	3	1·05	0·2309	0·2224	3·7	0·15
Mahua oil	3	1·45	0·2312	0·2235	3·3	0·65
Castor oil	3	0·80	0·2365	0·2269	4·1	...
Til oil ...	3	1·25	0·2352	0·2298	2·3	0·70
Linseed oil	3	1·00	0·2313	0·2294	0·8	0·80

TABLE V

Substance.	Amount of oil in c.c.	Amount of N/100 iodine in c.c. liberated before the oxidation of glucose.	Amount of CuO in grms. (blank).	Amount of CuO in grms. (after oxidation).	Percentage oxidized.	Amount of N/100 iodine in c.c. liberated after oxidation of glucose.
Mustard oil	3	0·90	0·2232	0·2168	2·9	0·2
Cocoanut oil	3	0·65	0·2208	0·2171	1·7	0·25
Mahua oil	3	0·85	0·2216	0·2176	1·8	0·40
Castor oil	3	0·50	0·2260	0·2229	1·4	0·15
Til oil	3	0·75	0·2251	0·2229	1·0	0·50
Linseed oil	3	0·65	0·2224	0·2211	0·6	0·50

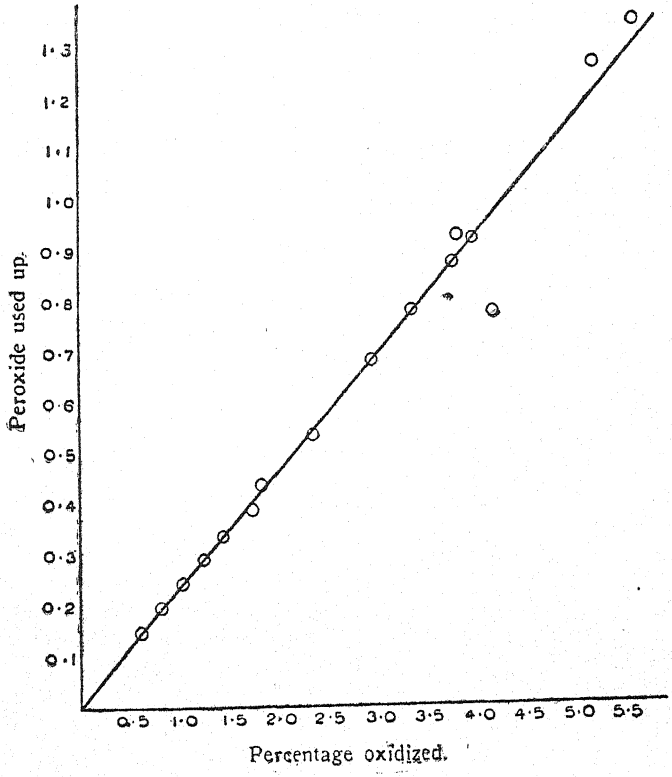
In the light of the observations made we can safely say that when the food-materials are exposed to sunlight in presence of air, they take up oxygen forming some peroxide type of compound which can oxidize other food-materials when mixed with them. Consequently, the addition of the exposed substances to ordinary food-stuff facilitates the proper ingestion of food-materials and produce efficacious results. From the above tables we find that mustard oil (*Brassica competris*) when exposed to light and air is capable of oxidizing glucose when mixed with it to the extent of 5 per cent. It is interesting to note that *Til* oil (oil of *Sesamum indicum*) has the least power of giving up oxygen from its peroxide and even after oxidizing glucose more than half of the peroxide remains unchanged as can be seen from the amount of iodine liberated by the oil after the oxidation of glucose. Thus, we see that the amount of oxidation of glucose is proportional to the amount of peroxide used up as shown in Table VI.

TABLE VI

Oils.	Amount of peroxide in terms of c.c. of N/100 iodine before oxidation of glucose.	Amount of peroxide in terms of c.c. of N/100 iodine after oxidation of glucose.	Peroxide used up during oxidation in terms of c c. of N/100 iodine.	Percentage oxidized.	$K=\frac{\text{Peroxide used up}}{\text{Percentage oxidized}}$
Mustard oil	1·50	0·20	1·30	5·1	0·255
Cocoanut oil	1·05	0·35	0·70	2·9	0·241
Mahua oil	1·10	0·15	0·95	3·9	0·244
Castor oil	0·95	...	0·95	3·8	0·250
Til oil	1·25	0·85	0·40	1·7	0·235
Linseed oil	1·0	0·7	0·30	1·2	0·250
Mustard oil	1·5	0·1	1·4	5·5	0·255
Cocoanut oil	1·05	0·15	0·9	3·7	0·243
Mahua oil	1·45	0·65	0·8	3·3	0·242
Castor oil	0·8	...	0·8	4·1	0·195
Til oil	1·25	0·70	0·55	2·3	0·239
Linseed oil	1·0	0·8	0·2	0·8	0·250
Mustard oil	0·9	0·2	0·7	2·9	0·241
Cocoanut oil	0·65	0·25	0·4	1·7	0·235
Mahua oil	0·85	0·40	0·45	1·8	0·250
Castor oil	0·5	0·15	0·35	1·4	0·250
Til oil	0·75	0·5	0·25	1·0	0·250
Linseed oil	0·65	0·5	0·15	0·6	0·250
Average	...	...	...	...	0·243

A glance at Table VI will show that except in a few cases, the ratio between the available oxygen from the peroxides of the oils for the oxidation of glucose and the percentage of glucose oxidized is fairly constant. It can also be seen from Fig. 1, that the graph showing the relation between the percentage of glucose oxidized and the amount of peroxide used up is a straight line. The average of the constants in Table VI is calculated to be 0·243. The constant as read from the graph comes out to be 0·241, *i.e.*, quite concurrent. The percentage of glucose oxidized by the peroxide of the oil is proportional to the availability of oxygen from the peroxide. It is not dependent upon the actual amount of peroxide formed. Mustard oil has thus the greatest efficiency in producing this type of oxidation.

Fig. 1.



Having investigated these facts on the efficiency of exposed oils in oxidizing other food-materials, we carried on experiments on metabolism of pigeons using these exposed and unexposed oils. Incidentally we have also investigated the influence of sunlight and small quantities of ferric chloride in the metabolism of pigeons.

Pigeons were divided amongst six compartments each containing four pigeons. The 1st set had sufficient amount of sunlight, the 2nd set had sunlight, to a much less extent, whereas all other sets were kept in darkness. The main diet was Rangoon rice which is believed to be entirely devoid of vitamins. Each of the pigeons got 25 grms. of Rangoon rice. In addition to that those in the 2nd set got 5 grms. of Bajra (*Pennisetum typhoideum*) for each pigeon. Those in the 3rd set got 3 c.c. of olive oil exposed to sunlight for 5 hours for four pigeons. The oil was well mixed with rice. The 5th set got 20 c.c. of 0·1 per cent ferric chloride solution for four pigeons. The 6th set got 3 c.c. of unexposed olive oil for four pigeons. The 1st and the 4th sets got only rice. This diet was continued for a month. All the pigeons steadily decreased in weight except those in the 2nd set which got Bajra and some sunlight. These pigeons were more or less normal in condition throughout the experiment. After two weeks the pigeons of the 4th set, *i.e.*, those receiving Rangoon rice in darkness showed stomachic trouble and one of the pigeons showed signs of polyneuritis. It could not move and had lost its appetite. It also lost its eyesight and was paralytic. The particular pigeon was separated from the rest of its batch and kept in sunlight for $2\frac{1}{2}$ hours. It improved a little and could walk slowly. The eyesight became normal but the appetite did not come back. It was then artificially fed with a little tomato-juice and milk. In about 18 hours it became almost normal. The diet was changed to Rangoon rice mixed with a little Bajra and the pigeon

was kept in light. Gradually it became quite normal in condition.

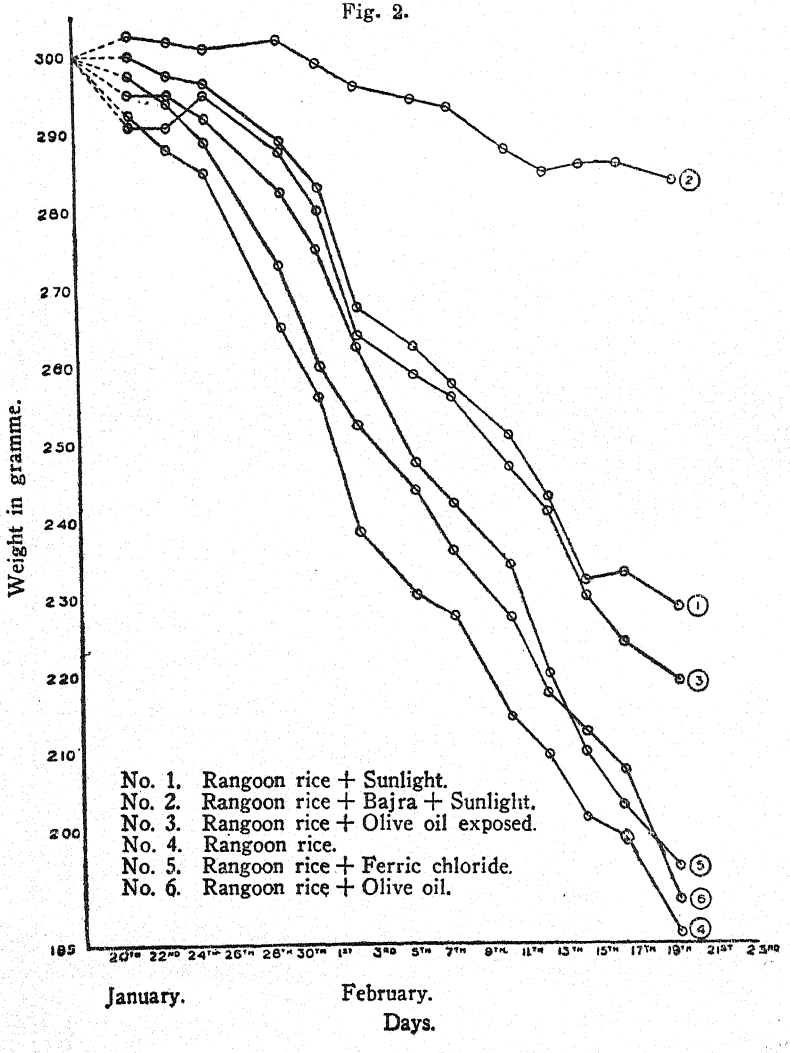
After 3 weeks the 5th and, at about the same time but a little later, the 6th set became affected with stomachic trouble. All the pigeons in these sets lost their appetite and the major portion of the food given was left untouched. The pigeons of the 4th set also had the same trouble but none of the other pigeons in the 1st, 2nd and 3rd sets showed any such trouble, though the pigeons in the 1st and 3rd sets were steadily decreasing in weight. In the fourth week one of the pigeons in the 5th set got affected with polyneuritis. It was kept in sunlight and was artificially fed with Bajra when it improved considerably but still was very weak. But it regained its appetite and could take a little rice mixed with tomato-juice and milk of itself. It gradually improved and became quite normal in 36 hours.

At the end of the fourth week, the eyes of the pigeons of the 4th set were greatly affected and majority of the pigeons in the 5th and 6th sets were seriously ill. In one night one pigeon of the 5th set and two of the 6th set became so much affected with the disease that they were beyond the power of treatment, and all of them died before any food could be administered. In about five hours another pigeon of the 5th set became affected and in about an hour the case became very serious. It was then artificially fed with tomato-juice and milk. When it improved, the diet was changed to Bajra. But after two days it had a relapse and died. After four weeks of experiment, the normal diet was resumed and all the pigeons were kept in well-lighted compartments. But on the same day one of the pigeons in the 4th set showed slight signs of paralysis. In the night the case became serious and in the morning it was dead.

During the experiment all the pigeons were weighed on alternate days. In order to compare the fall in weight

and the general condition of the pigeons, the average weight of each set every day was calculated, taking 300 as the original weight of each set and a graph was plotted. The curve in Fig. 2 clearly shows that the 2nd set which received Bajra were more or less in normal condition. The 1st set which received sunlight and the 3rd set which received olive oil exposed to sunlight were almost similar in condition. The 5th and 6th sets were almost equally affected but the 4th set was the most affected.

Fig. 2.



Our results show that animals receiving normal diet and sunlight keep very good health. Even if the animals do not get any vitaminous food but only sunlight, they keep good health as the pigeons in the 1st set. Of course the pigeons which got olive oil exposed to sunlight were much better than those getting iron and unexposed olive oil though they were kept in darkness, still they were inferior in general health to those which obtained sunlight but no vitaminous food. We thus find that olive oil exposed to sunlight is not as efficacious as the vitaminous food or even as sunlight. In a previous paper it has been shown that iron in small doses such as are present in leafy vegetables is beneficial to health. Our results show that iron in larger doses is rather harmful to the pigeons and cannot prevent the attack of polyneuritis. Ordinarily olive oil also is not a nutritive diet for pigeons and its presence does not help them in preventing the disease. These experiments confirm the view that sunlight acts as a promoter of oxidation of the food-materials in the body and normal food with plenty of sunlight is the best kind of diet.

SUMMARY

1. It has been shown that if olive, mustard, cocoanut, mahua, castor, til, and linseed oils, butter and some carbohydrates are exposed to sunlight, and air is passed through them, peroxides are formed. These peroxides have been estimated by the amount of iodine liberated by them from an acid solution of potassium iodide.

2. If the oils are only exposed to sunlight and air is not passed through them, the amount of peroxides is decreased to a great extent. The amount of peroxide formed is due to the layer of air which comes in contact with the oil surface.

3. All these oil-peroxides have the property of oxidizing a solution of glucose when mixed with them and kept at 40°C. for 18 hours. The amount of this oxidation is dependent upon the availability of oxygen from the peroxide.

4. From the experiments on metabolism of pigeons using exposed and unexposed oils, iron and sunlight, we have proved that sunlight is the best preventive for diseases like polyneuritis and beri-beri. Olive oil exposed to sunlight and air comes a close second. Whereas iron and unexposed oils are harmful to these pigeons.

5. The natural food with plenty of sunlight seems to be the best kind of diet for the maintenance of health.

SECTION III

BOTANY

THE FUNGUS FLORA OF ALLAHABAD

PART II

BY

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A list of the fungi of Allahabad was published by Saksena, K. L., in 1927. His observations extended over only a few months and a number of other fungi have since been collected by the authors who are therefore publishing this supplementary list as Part II of "The Fungus Flora of Allahabad."

It must be made clear that our collections were limited to the fields, gardens and parks of the town itself: it was not found possible to tour in the district. The area under review is thus very limited in extent.

The localities given for the various fungi in the list refer only to the first observations, the same fungus having in many cases been found in other localities also. A few fungi apparently collected and identified by Mr. K. L. Saksena do not appear in his list. These are included in our list and marked with an asterisk. The Eu-Basidiomycetes of Allahabad of which many sprout up during the rains are not included in this publication. This is partly due to the fact that the 1928 rains were poor and the mushroom crop was consequently not rich in new forms. The few Eu-Basidiomycetes we did collect have already been described by K. L. Saksena. Considerable difficulty

continues to be experienced in naming the specimens collected. The literature available in the department is still scanty and even a visit to Pusa by one of the authors did not enable him to fully name all the specimens. Many of the fungi have therefore been run down only to the genus.

We wish to express our indebtedness to Dr. William McRae, M.,A., D.Sc. (London), Imperial Mycologist, Pusa, for his liberality in placing his herbarium at our disposal. We have also to thank Mr. S. N. Mitra and Mr. R. R. Sen of the Mycological Section, Pusa, and Mr. Udaibhan Singh, Research Scholar, Allahabad University, for their kind help. We must acknowledge the assistance given by Mahadeo, Head Mali of the Botanical Garden, who collected some very valuable specimens.

No.	Fungus.	Habitat.	Locality.	Date of Collection.
		PHYCOMYCETES		
1	Peronospora parasitica (Pers.) De By.	Brassica campestris Var. Sarson.	George Town	23-2-'28
2	Peronospora vicae (Berk.) de Bary.	Pisum arvense	Fort Road	1-2-'29
3	Peronospora arborescens (Berk.) de Bary.	Argemone mexicana ...	Fort Road	1-2-'29
4	Peronospora effusa (Grev.) Rbh.	Chenopodium album ...	Near Bharatdwaj ...	18-1-'29
5	Peronospora sp.	Spergula arvensis ...	University Botanical Garden	28-1-'29
6	Peronospora sp.	Trigonella Foenum-graecum	University Botanical Garden	15-1-'29
7	Peronospora sp.	Brassica oleracea ...	Bund Road	10-11-'27
8	Cystopus candidus (Pers.) Lev.	Brassica campestris Var. Sarson.	Munfortgunj	25-2-'29
9	Cystopus platensis speg. ...	Boerhaavia diffusa ...	University Botanical Garden	18-8-'28
10	Cystopus Ipomoeae-panduratae (Schw.) stev et swing.	Ipomoea reptans ...	University Botanical Garden	20-8-'28

No.	Fungus.	Habitat.	Locality.	Date of Collection.
		PHYCOMYCETES—(*contd.*)		
11	Rhizopus artocarpi Rac. ...	Artocarpus integrifolia ...	University Botanical Garden	3-9-'28
12	Rhizopus sp.	Ficus carica	University Botanical Garden	7-1-'29
		ASCOMYCETES		
13	Balansia sp.	Panicum javanicum ...	Bund Road	
14	Botryosphaeria Agaves P. Henn.	Agave americana ...	Bund Road	2-1-'29
15	Capnodium sp.	Bombax malabaricum ...	University Botanical Garden	3-12-'27
16	Capnodium sp.	Unidentified	Alfred Park	16-12-'27
17	Catacauma Acaciae Thiess. et Syd.	Acacia leucophlaea .	McPherson Lake ...	7-12-'28
18	Erysiphe cichoracearum Dc. ...	Coccinia indica	Kutcherry Road ...	28-9-'27
19	Erysiphe graminis Dc. ...	Triticum vulgare ...	Field along Fort Road ...	25-2-'29
*20	Erysiphe Polygoni Dc. ...	Melilotus alba	University Botanical Garden	
21	Glomerella sp.	Carica papaya	Khusrobagh	8-12-'27
22	Phyllachora cynodontis (Sacc.) Niessl.	Cynodon dactylon ...	University Botanical Garden	10-12-'27

23	Phyllactinia corylea (Pers.) Karst.	Dalbergia sissoo... ...	McPherson Lake ...	6-12-'28
		URED INAE		
24	Hapalophragmium ponderosum Syd. et Butl.	Acacia leucophlaea ...	Mindhauri	25-12-'27
25	Melampsora helioscopiae (Pers.) Cas.	Euphorbia-dracunculoides ...	Muir Road	31-3-'28
26	Puccinia butleri Syd. ...	Launea asplenifolia ...	Shiv Kuti	
27	Puccinia ruelliae (B. et Br.) Lagh.	Ruellia prostrata ...	Wanamaker Girls' School ...	23-12-'27
28	Puccinia romagnoliana Maire. and Sacc.	Cyperus rotundus ...	University Botanical Garden	9-12-'27
29	Uromyces Hobsoni Vize aecidial stage.	Jasminum arborescens ...	Near Holy Trinity Church ...	25-8-'27
30	Uromyces Hobsoni Vize Uredo and Teleuto stage.	Jasminum arborescens ...	Near Holy Trinity Church ...	25-8-'27
31	Uromyces leptodermus Syd. ...	Panicum javanicum ...	University Botanical Garden	
32	Uromyces fabae (Pers.) de Bary.	Pisum arvense... ...	Naini	25-2-'28

No.	Fungus.	Habitat.	Locality.	Date of Collection.
		USTILAGINAE		
33	Ustilago tritici (Pers.) jens.	Triticum vulgare ...	Near Bharatdwaj ...	22-2-'29
34	Ustilago operta Syd. et Butl ...	Panicum javanicum ...	University Botanical Garden	15-10-'27
		FUNGI IMPERFECTI		
35	Alternaria Brassicae (Berk.) Sacc.	Brassica campestris Var. Sarson.	Beli Road	6-3-'27
36	Alternaria Brassicae (Berk.) Sacc.	Brassica oleracea ...	Bund Road	12-1-'28
37	Alternaria Brassicae (Berk.) Sacc.	Raphanus sativus ...	Naini	22-2-'28
38	Alternaria sp.	Dracaena sp.	University Botanical Garden	
39	Ascochyta sp.	Artocarpus integrifolia ...	A Garden	4-1-'28
40	Cercospora ricinella Sacc. and Berk	Ricinus communis ...	Bund Road	10-11-'27
41	Cercospora subsessilis Syd. ...	Melia Azedarach ...	University Botanical Garden	8-11-'28

42	Cercospora sp.	Porana paniculata ...	Beli Road	5-1-'28
43	Cercospora sp. ...	Cajanus indicus ...	Beli Road ...	5-1-'28
44	Cercospora sp. ...	Cordia myxa ...	Alfred Park ...	21-1-'27
45	Cercospora sp. ...	Feronia elephantum ...	Bund Road ...	10-12-'27
46	Cercospora sp. ...	Unknown compositae ...	Khusrobagh ...	8-12-'27
*47	Colletotrichum Lineola Corda...	Andropogon sorghum ...	...	15-10-'26
48	Colletotrichum sp. ...	Carica papaya ...	Khusrobagh ...	8-12-'27
49	Cladosporium sp. ...	Caesalpinia Bonducella ...	University Botanical Garden	11-11-'27
50	Cladosporium sp. ...	Porana paniculata ...	Beli Road ...	6-3-'27
51	Cladosporium sp. ...	Clerodendron sp. ...	Katra Housing Scheme ...	5-1-'28
52	Cladosporium sp. ...	Cassia obtusifolia ...	Allahabad ...	11-12-'27
53	Cladosporium sp. ...	Sesbania aculeata ...	University Botanical Garden	24-10-'27
54	Cladosporium sp. ...	Sesamum indicum ...	Jumna Bank ...	28-11-'27
*55	Cladosporium sp. ...	Cucurbita moschata ...	Bund Road ...	10-11-'26
56	Diplodia-dracaenicola (Died) ...	Dracaena ..	University Botanical Garden	28-11-'28

No.	Fungus.	Habitat.	Locality.	Date of Collection.
57	Exosporium sp. ...	Dalbergia sissoo ...	Alfred Park ...	5-12-'28
58	Fusarium Limonis Briosi ...	Citrus Medica Var. Limonis (Lemon).	Khusrobagh ...	8-12-'27
59	Fusarium sp. ...	Capparis sepiaria ...	Near Holy Trinity Church ...	27-9-'27
60	Fusarium sp. ...	Cassia obtusifolia ...	Mayo Road ...	11-12-'27
61	Fusarium sp. ...	Artocarpus integrifolia ...	University Botanical Garden	12-1-'29
*62	Fusarium sp. ...	Sorghum vulgare ...	...	28-10-'26
*63	Fusarium sp. ...	Cucurbita Moschata ...	Bund Road ...	10-11-'26
*64	Fusarium sp. ...	Pennisetum tryphoideum ...	...	10-10-'26
65	Fumago sp. ...	Hibiscus esculentus ...	Khusrobagh ...	8-12-'27
66	Graphiola phoenicis (Mong) Poit.	Phoenix sylvestris ...	Alfred Park ...	6-12-'28
*67	Helminthosporium turcicum Pass.	Sorghum vulgare ...	...	28-10-'26
*68	Helminthosporium sp. ...	Pennisetum typoideum ...	...	10-10-'26
69	Macrophoma Dianthi Gabotto...	Dianthus Chinensis ...	University Botanical Garden	22-9-'28

70	Macrosporium sp. ...	Nelumbium sp. ...	University Botanical Garden	11-11-'27
71	Macrosporium sp. ...	Cajanus indicus ...	Beli Road ...	5-1-'28
72	Macrosporium sp. ...	Brassica campestris Var. sarson.	Naini ...	25-2-'27
73	Macrosporium sp. ...	Sesbania aculeata ...	University Botanical Garden	24-10-'27
74	Macrosporium sp. ...	Sesamum indicum ...	Jumna Bank ...	28-11-'27
75	Macrosporium sp. ...	Rosa sp. ...	University Botanical Garden	22-2-'29
76	Oidiopsis taurica (Lev.) Salmon	Tephrosia tenuis ...	A Garden ...	4-1-'28
77	Oidium erysiphoides Fr. ...	Coccinia indica ...	Bank Road ...	30-11-'27
78	Oidium erysiphoides Fr. ...	Euphorbia sp. ...	Khusrobagh ...	8-12-'27
79	Oidium erysiphoides Fr. ...	Zizyphus jujuba ...	Khusrobagh ...	8-12-'27
80	Oidium erysiphoides Fr. ...	Pisum arvense ...	Naini ...	
81	Oidium erysiphoides Fr. ...	Papaver rhoeas ...	University Botanical Garden	
82	Oidium erysiphoides Fr. ...	Lathyrus sativus ...	University Botanical Garden	
83	Phyllosticta clerodendri Syd et Butl.	Clerodendron sp. ...	New Katra ...	5-1-'28
84	Ramularia sp. ...	Peristrophe bicalyculata ...	University Botanical Garden	6-11-'28

SECTION IV

MATHEMATICS

ON THE NON-PERIODIC SOLUTIONS OF THE DIFFERENTIAL EQUATION

$$\frac{d^2y}{dx^2} + (A + 16\ q \cos\ 2nx)y = 0$$

BY

RAMA SHANKAR VARMA, M.Sc.

In a recent paper,* the writer has found the periodic solutions of the differential equation

$$\frac{d^2y}{dx^2} + (A + 16\ q \cos\ 2nx)\ y = 0, \quad \ldots \quad \ldots \quad (1)$$

where

n is a given integer,

q is a given quantity so small that its higher powers may be neglected,

and $A = m^2 + a^1$, m being an arbitrary integer† including zero and a^1 a function of m and q, which tends to zero as q tends to zero and which is chosen in such a way that one solution of (1) is periodic.

The object of this paper is to obtain the second solution of the differential equation (1) for the values of A which give rise to the periodic solutions considered in the previous paper. We know that these solutions are non-periodic.‡

In analogy with the notation for the second kind of solution of Mathieu's equation, let us denote the solutions of the rth order, corresponding to $c_n e_r\ (x, q)$ and $s_n e_r\ (x, q)$, by

$$i_n \eta_r\ (x, q) \text{ and } j_n \eta_r\ (x, q),$$

respectively.

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* See, the *Journal of Indian Mathematical Society*, Vol. XVIII.

† m denotes the order of the solution.

‡ Lindsey Ince, Proc. Edin. Math. Soc., Vol. XXXIII (1915).

It is obvious, that, as there are $(n-1)$ solutions between such solutions $c_n e_r(x, q)$ as give rise to Mathieu functions by putting $n=1$, there must be $(n-1)$ solutions $i_n\eta_r(x, q)$ between the two which give Mathieu functions of the second kind by putting $n=1$. A similar statement is true for the solutions $j_n\eta_r(x, q)$.

We shall first obtain $i_n\eta_r(x, q)$. The value of A for this solution is given by

$$A = r^2 - \frac{32}{n^2-r^2}q^2 + \ldots \quad \ldots \quad \ldots \quad (2)$$

$$= a_0 + a_2q^2 + \ldots\ldots(\text{say}) \quad \ldots \quad \ldots \quad (3)$$

Now equation (1) can be written as

$$\frac{d^2y}{dx^2} + r^2y = -(a' + 16q\cos 2nx)y \quad \ldots \quad (4)$$

so that

$$a' = a_2q^2 + \ldots\ldots \quad \ldots \quad \ldots \quad (5)$$

Next suppose that

$$y(x) = \psi_0(x) + q\psi_1(x) + q^2\psi_2(x) + \ldots \quad \ldots \quad (6)$$

where ψ_0, ψ_1, ψ_2.....do not contain q.

Now $y(x)$ is given by the integral equation*

$$y(x) = B\sin rx + \frac{1}{r}\int_0^x (a' + 16\, q\cos 2nt)\sin r(t-x)y(t)\,dt$$ where B is an arbitrary constant ... (7)

Put

$$B = 1 + b_1q + b_2q^2 + \ldots\ldots$$

Substituting in the integral equation (7) the value of a' and $y(x)$ as given by (5) and (6), and equating the like powers of q, we get

$$\psi_0(x) = \sin rx$$

$$\psi_1(x) = b_1\sin rx + \frac{1}{r}\int_0^x 16\cos 2nt\,\sin r(t-x)\psi_0(t)\,dt,$$

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* S. C. Dhar, Bulletin Cal. Math. Soc., Vol. XVIII (1927).

$$\psi_2(x) = b_2 \sin rx + \frac{a_2}{r}\int_0^x \sin r\,(t-x)\,\psi_0\,(t)\,dt$$

$$+\frac{1}{r}\int_0^x 16\cos 2nt \sin r\,(t-x)\psi_1\,(t)\,dt.$$

. . . . . .

On solving the above equation in succession, we obtain $\psi_1, \psi_2, \ldots\ldots$ The numerical constants $b_1, b_2 \ldots\ldots$ are obtained by imposing the condition that the expressions for $\psi_1, \psi_2 \ldots\ldots$ must not contain sin rx.

Thus we obtain

$$\psi_1\,(x) = \left(b_1 + \frac{4}{n^2-r^2}\right)\sin rx + \frac{2\sin(2n+r)x}{n\,(n+r)} - \frac{2\sin(2n-r)x}{n\,(n-r)}$$

Putting $b_1 = -\dfrac{4}{n^2-r^2}$,

$$\psi_1\,(x) = \frac{2\sin(2n+r)x}{n\,(n+r)} - \frac{2\sin(2n-r)x}{n\,(n-r)}.$$

With this value of $\psi_1\,(x)$, we have

$$\psi_2\,(x) = \left[b_2 + \frac{4\,(28n^2-r^2)}{n^2\,(n^2-r^2)\,(4n^2-r^2)}\right]\sin rx + \frac{2\sin(4n+r)\,x}{n^2\,(n+r)\,(2n+r)} - \frac{2\sin(4n-r)\,x}{n^2\,(n-r)\,(2n-r)}$$

Thus we see that

$$i_n\,\eta_r\,(x, q) = \sin rx + q\left\{\frac{2\sin(2n+r)x}{n\,(n+r)} - \frac{2\sin(2n-r)x}{n\,(n-r)}\right\}$$

$$+ q^2\left\{\frac{2\sin(4n+r)\,x}{n^2(n+r)(2n+r)} - \frac{2\sin(4n-r)\,x}{n^2\,(n-r)\,(2n-r)}\right\} + \ldots \quad (8)$$

We notice that the above solution breaks down when n is a sub-multiple of r because the denominators in some of the terms vanish. This difficulty does not arise if we start afresh and obtain the solutions for these particular cases direct from the integral equation (7). We give below a few such cases.

For $i_n\,\eta_n\,(x, q)$

$$a^1 = -8q - \frac{8}{n^2}q^2 + \ldots,$$ giving

$$\psi_0(x)=\sin nx$$

$$\psi_1(x)=\frac{\sin 3nx}{n^2}-\frac{8}{n}x\cos nx$$

$$\psi_2(x)=\frac{5\sin 3nx}{n^4}+\frac{\sin 5nx}{3n^4}-\frac{8}{n^3}x\cos 3nx.$$

Hence

$$i_n\eta_n(x,q)=\sin nx+q\left\{\frac{\sin 3nx}{n^2}-\frac{8}{n}x\cos nx\right\}$$

$$+q^2\left\{\frac{5\sin 3nx}{n^4}+\frac{\sin 5nx}{3n^4}-\frac{8}{n^3}x\cos 3nx\right\}+\ldots$$

Similarly

$$i_n\eta_{2n}(x,q)=\sin 2nx+q,\frac{2\sin 4nx}{3n^2}$$

$$+q^2\left\{x\frac{8\cos 2nx}{3n^3}+\frac{\cos 6nx}{6n^4}\right\}+\ldots$$

As the solution $i_n\eta_0(x,q)$ must tend to x when q tends to zero, we notice that the method followed above fails to give the function. We shall, hence, take to a different method for deducing the solution.

Let us take the form of the function to be

$$y=fo(x)+qf_1(x)+q^2f_2(x)+\ldots.$$

The value of A for which this is to hold is

$$A=-\frac{32q^2}{n^2}+224\frac{q^4}{n^n}+\ldots.$$

Substituting the value of y and A in (1), we have

$$fo''(x)+qf_1''(x)+q^2f_2''(x)+\ldots$$

$$+\left\{-32\frac{q^2}{n^2}+\ldots.16q\cos 2nx\right\}$$

$$\times\left\{fo(x)+qf_1(x)+q^2f_2(x)+\ldots\right\}=0$$

Equating the different powers of q to zero, we get the following differential equations from which to determine $fo(x)$, $f_1(x)$, $f_2(x)$ .... etc.

$$\left.\begin{aligned}fo''(x)&=0\\f_1''(x)&=-16\cos 2nx\,fo(x)\\f_2''(x)&=\frac{32}{n^2}fo(x)-16\cos 2nx\,f_1(x)\end{aligned}\right\}\ldots\quad\ldots\quad\ldots\quad(9)$$

Integrating the first of the above equations, we have

$$fo\,(x) = c_1 x + c_2,$$

where c_1 and c_2 are constants.

Since we have decided to make the coefficient of x to be unity, we choose

$$c_1 = 1, \quad c_2 = 0$$

This gives

$$fo\,(x) = x.$$

In sloving the other equations of (9), we will introduce no terms in x, since we decide to normalize the function $i_n\eta_o$ (x, q) by assuming that the coefficient of x in it is strictly unity. Thus we have

$$f_1\,(x) = \frac{4}{n^2}\, x \cos 2nx - \frac{4}{n^3} \sin 2nx$$

$$f_2\,(x) = \frac{2}{n^4}\, x \cos 4nx - \frac{3}{n^5} \sin 4nx.$$

Hence we have

$$i_n\eta_o\,(x, q) = x + q\left\{\frac{4}{n^2}\, x \cos 2nx - \frac{4}{n^3} \sin 2nx\right\}$$

$$+ q^2\left\{\frac{2}{n^4}\, x \cos 4nx - \frac{3}{n^5} \sin 4nx\right\} + \ldots$$

$$= x\; c_n e_o\;(x,\; q) - \frac{4q}{n^3} \sin 2nx$$

$$- \frac{3q^2}{n^5} \sin 4nx + \ldots\ldots$$

The solutions $j_n\eta_r\,(x,\ q)$, $j_n\,\eta_{kn}$ $(x,\ q)$ can be deduced in a similar manner.

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